AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS NUMBER

Vol. XIV FEBRUARY, 1929 No. 5

The Journal of Laboratory and Clinical Medicine

Medical Library

FEB 26 1929

WARREN T. VAUGHAN, M.D., EDITOR
Medical Arts Building
Richmond, Va.

ASSOCIATE EDITORS

Pharmacology
DENNIS E. JACKSON, M.D.
University of Cincinnati, Cincinnati

Pathology
PAUL G. WOOLLEY, M.D.
Pasadena

Physiology
PROFESSOR J. J. R. MACLEOD
Physiological Laboratory, Marischal College, Aberdeen, Scotland

Tuberculosis
GERALD B. WEBB, M.D.
Cragmor Sanatorium, Colorado Springs

Clinical Pathology
W. C. MacCARTY, M.D.
Mayo Clinic, Rochester, Minn.

HAROLD D. CAYLOR, M.D.
Mayo Clinic, Rochester, Minn.

Biochemistry
VICTOR C. MYERS, Ph.D.
Western Reserve University, Cleveland

Experimental Medicine
RUSSELL L. HADEN, M.D.
University of Kansas, Rosedale

Immunology
JOHN A. KOLMER, M.D.
University of Pennsylvania, Philadelphia

ROBERT A. KILDUFFE, M.D.
Atlantic City, N. J.

Bacteriology
A. PARKER HITCHENS, M.D.
Bureau of Science, Manila, P. I.

Surgery
DEAN LEWIS, M.D.
Johns Hopkins University, Baltimore

Internal Medicine
GEORGE HERRMANN, M.D.
Tulane University, New Orleans

T. B. MAGATH, M.D.
Editor for the American Society of Clinical Pathologists

Official Organ of the American Society of Clinical Pathologists and the American Association for the Study of Allergy.

PUBLISHED BY THE C. V. MOSBY COMPANY, 3523-25 PINE BLVD., ST. LOUIS.
Entered at the Post Office at St. Louis, Mo., as Second Class Matter.

CORAMINE, "CIBA"

WHEN STRYCHNINE, CAFFEINE AND CAMPHOR FAIL

CIBA COMPANY, Inc., NEW YORK CITY

Also see lower half of page 2

FREAS

ELECTRIC HEAT AUTOMATICALLY CONTROLLED

INCUBATORS

meet the demands of

Modern Practice

TIME saving, dependable, automatic—Freas incubators relieve busy professional men of the necessity of attending a routine process; safeguard valuable cultures; automatically maintain constant temperatures for bacteria incubation; and expedite paraffin work. They are electrically heated, and equipped with the Freas patented regulator. Quality construction is used throughout; walls are well insulated; shelves are adjustable; inner glass door permits inspection of contents without exposure; and pilot light, externally operated, illuminates the chamber for observations. Freas incubators are furnished complete, with connecting cord and plug, with table base or floor stand.

On display in the showrooms of our distributors listed below—call upon them for a demonstration of the Freas regulator and other features—or write to us for illustrated literature.

FREAS THERMO-ELECTRIC COMPANY

1215 SOUTH GROVE STREET

IRVINGTON, N. J.

DISTRIBUTORS OF FREAS LABORATORY OVENS AND INCUBATORS

Elmer & Amend, *New York, N. Y.*
Will Corporation, *Rochester, N. Y.*
Fisher Scientific Company, *Pittsburgh, Pa.*
E. H. Sargent & Co., *Chicago, Ill.*
Doster-Northington Co., *Birmingham, Ala.*
Braun-Knecht-Heimann Co., *San Francisco, Calif.*
Braun Corp., *Los Angeles, Calif.*
Kansas City Laboratory Supply Co., *Kansas City, Mo.*
Denver Fire Clay Co., *Denver, Colo.*
Canadian Laboratories Supplies, Ltd., *Toronto 2, Canada*
Fisher Scientific Co., Ltd., *Montreal, Canada*
Antiga & Company, *Havana, Cuba*
H. V. Groach Co., *San Juan, Porto Rico*
Tanaka Shoji Kaishia, Ltd., *Tokio, Japan*
A. Gallenkamp & Co., Ltd., *London, E. C. 2 England*

The Journal of Laboratory and Clinical Medicine

VOL. XIV ST. LOUIS, MO., FEBRUARY, 1929 NO. 5

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

FURTHER OBSERVATIONS WITH A NEW METHOD FOR CULTIVATING TUBERCLE BACILLI: A COMPARISON WITH GUINEA PIG INOCULATION AND PETROFF'S METHOD*

BY H. J. CORPER, M.D., PH.D., AND NAO UYEI, PH.D., DENVER, COLORADO

PART I

UP TO the present time two methods have surpassed culture methods for the detection of tubercle bacilli in the experimental and clinical study of tuberculosis. The microscopic examination for stained bacilli in smears or sections is the most favored, probably because of its ease of manipulation, but it lacks the delicacy of the more expensive guinea pig inoculation method. In the experimental laboratory the culture method has proved itself adaptable to many uses and a valuable means for isolating bacilli, thus making it unreplaceable by either staining methods or animal inoculation. The satisfaction of watching a growth of bacilli develop on the culture medium is gratifying especially in comparison with any method of animal inoculation. Since, however, culture methods in the past merited neither speed nor delicacy it was hardly to be expected that they would come in general practical use. In earlier publications,[1] however, a new method was described by us for the isolation of tubercle bacilli from contaminated tuberculous materials which in preliminary tests fulfilled the requirements necessary to suggest the possibility of its use as a substitute for the guinea pig inoculation method. At that time it was reported that of the various reagents used for the primary isolation of tubercle bacilli from contaminated tuberculous materials, sulphuric acid and hydrochloric acid were found superior to any other reagents tried, including sodium hydroxide, for destroying the undesirable contami-

*Research Department, National Jewish Hospital at Denver, Colorado.

Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minnesota, June 8, 9, and 11, 1928.

nators present. Standard crystal violet or methyl violet of the American dyes was found serviceable in replacing the prewar Grübler's gentian violet, and in the same concentration, for preparing Petroff's gentian violet egg medium. Crystal violet potato cylinder medium was found superior to gentian violet egg medium, Dorset's egg medium, Petroff's gentian violet egg medium, glycerol-agar and Long's nonprotein medium, in favoring the growth of the bacilli when present in small numbers. The other media were found serviceable in this respect in the order given above. The new method for the isolation of tubercle bacilli consisted in using an equal volume of 6 per cent sulphuric acid at 37° C. for thirty minutes in the preliminary treatment of the tuberculous material, and crystal violet potato cylinder medium for culturing the bacilli. The crystal violet potato medium was prepared by immersing clean potato cylinders in 0.0015 per cent standard crystal violet in a 1 per cent solution of sodium carbonate for one to two hours and autoclaving the potato cylinders for thirty minutes at 15 pounds pressure after placing in a sterile culture tube containing 1.5 c.c. of 5 per cent glycerol broth. Since the appearance of our earlier reports, we have been engaged in attempts to simplify the medium and to evaluate the new method further in practical tests with sputums, urines, and animal tissues. The following report will be concerned with the findings obtained thus far in these studies.

ATTEMPTS TO IMPROVE AND SIMPLIFY THE CRYSTAL VIOLET POTATO CYLINDER GLYCEROL BROTH MEDIUM

Since the original potato cylinder medium containing the 1.5 c.c. of glycerol broth did not appear to us to be as convenient as an entirely solid medium for isolating tubercle bacilli, an attempt was made to prepare a medium in which ground potato was incorporated with agar-agar and in which other ingredients were used in varying proportions for the purpose of learning the most efficient combinations suited to the purpose of supporting the growth of the tubercle bacilli.

The first experiments in this series were concerned with finding the most suitable combination of ground potato, glycerol, and broth in agar-agar for enhancing the growth of tubercle bacilli.

a. *The Optimum Proportion of Ground Potato in Glycerol Agar Mediums for the Growth of Tubercle Bacilli.*—A standard medium ("S") consisting of 13.4 per cent of 4 times ordinary strength (4x) broth (containing 12 grams of beef extract, 40 grams of peptone, and 20 grams of sodium chloride diluted to 1000 c.c. with tap water), 2.0 per cent pure glycerol, and 1.1 per cent agar-agar were mixed with different proportions of freshly ground potato (ranging from 5 to 60 per cent) and distilled water. After mixing well, the medium was neutralized by means of 10 per cent sodium carbonate (c. p. anhydrous) solution using litmus paper as a test. The mixture was then sterilized in an autoclave at 15 pounds pressure for thirty minutes (excessive heating was avoided as this has been found to have a destructive influence upon the growth promoting properties of the potato for tubercle bacilli). In order to test the efficiency of these mixtures for supporting the growth of tubercle bacilli, the tubes of medium were seeded with three different strength sus-

persions of virulent human tubercle bacilli (Gluckson), containing 1 mg., 0.000,1 mg. or 0.000,001 mg. of bacilli per cubic centimeter.

The results of these cultural studies after four weeks with the 1.0 mg. per c.c. seeding, six weeks after the 0.000,1 mg. per c.c. seeding, and ten weeks after the 0.000,001 mg. seeding are recorded in Table I using as control the potato cylinder medium without crystal violet.

TABLE I

THE GROWTH OF VIRULENT HUMAN TUBERCLE BACILLI ON GLYCEROL AGAR NUTRIENT MEDIUM CONTAINING DIFFERENT AMOUNTS OF GROUND POTATO

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG. PER C.C. IN SUSPENSION USED FOR SEEDING CULTURE MEDIUM		
	1.0	0.000,1	0.000,001
Control: Potato cylinder medium without crystal violet	3*	2	1
"S" (standard glycerol agar broth medium) + 60% ground potato	3	2	0
"S" + 30% ground potato	4	3	2
"S" + 20% ground potato	3	2	2
"S" + 10% ground potato	2	2	1
"S" + 5% ground potato	1	1	0

*The arbitrary grading from 0 to 4 indicates the approximate amount of growth obtained from an average of 10 or more tubes seeded in each case and in most cases the experiment was duplicated a second time. In the 1.0 column the readings recorded are after four weeks incubation, in the 0.000,1 mg. column after eight weeks, and in the 0.000,001 mg. column after ten weeks incubation.

The findings recorded in Table I make it evident that tubercle bacilli grow best on a medium containing, in addition to the usual ingredients of 5 per cent glycerol broth agar, about 30 per cent ground potato. On this medium a better growth was obtained than on the plain broth potato cylinder medium without crystal violet, which would seem to require elucidation in view of the fact that this medium is not selected in the final analysis when choosing a method for isolating tubercle bacilli. The explanation lies in the fact, however, that crystal violet incorporated in the 30 per cent ground potato glycerol broth agar medium exerted a far more deleterious influence on the growth of the tubercle bacilli than that used with the cylinder medium.

b. *The Glycerol Concentration in the Medium.*—Since in isolating tubercle bacilli, especially when present in small numbers, any slight variation in composition of medium may mean a diminished power of the bacilli to grow, it was deemed advisable to test all the components of the medium including the glycerol: Therefore varying amounts from 1 to 5 per cent were added to a basic medium (B) consisting of ground potato 20 per cent, broth (4x) 13.4 per cent and agar 1.1 per cent. The culture medium was neutralized to litmus by means of a 10 per cent solution of sodium carbonate (c. p. anhydrous) and it was then sterilized by autoclaving for thirty minutes at 15 pounds pressure. The results of this study are recorded in Table II.

It is to be noted from the data recorded in Table II that with heavier seeding the virulent human tubercle (Gluckson) bacilli grew best on the ground potato broth agar medium containing 4 per cent glycerol while after lighter seeding (0.000,001 mg. per c.c.) the medium containing 2 to 2½ per cent of glycerol proved more suitable.

This experiment was duplicated, using, in place of the human bacilli, a virulent strain of bovine tubercle bacilli and the findings are recorded in Table III.

The findings in Table III indicate that virulent bovine tubercle bacilli with heavier seeding (1 mg. per c.c.) grew best in the potato broth agar medium containing 4 per cent glycerol while after seeding with small numbers of bacilli they grew best with a concentration of 2.0 to 2.5 per cent of glycerol.

TABLE II

THE GROWTH OF VIRULENT HUMAN TUBERCLE BACILLI ON THE POTATO BROTH AGAR MEDIUM CONTAINING VARYING AMOUNTS OF GLYCEROL

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG. PER C.C. IN SUSPENSION USED FOR SEEDING CULTURE MEDIUM		
	1.0	0.000,1	0.000,001
Control: Potato cylinder broth medium without crystal violet	3*	2	1
"B" (potato broth agar medium) + 5% glycerol	3	3	1
"B" + 4% glycerol	4	3	1
"B" + 3% glycerol	3	3	1
"B" + 2½% glycerol	3	3	2
"B" + 2% glycerol	3	3	2
"B" + 1% glycerol	1	2	1

*The arbitrary grading from 0 to 4 indicates the approximate amount of growth obtained from an average of 10 or more tubes seeded in each case. In the 1.0 column the readings recorded are after four weeks incubation, in the 0.000,1 mg. column after eight weeks, and in the 0.000,001 mg. column after ten weeks incubation.

TABLE III

THE GROWTH OF VIRULENT BOVINE TUBERCLE BACILLI ON THE POTATO BROTH AGAR MEDIUM CONTAINING VARYING AMOUNTS OF GLYCEROL

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG. PER C.C. IN SUSPENSION USED FOR SEEDING CULTURE MEDIUM		
	1.0	0.000,1	0.000 001
Control: Potato cylinder broth medium without crystal violet	2*	1	1
"B" (potato broth agar medium) + 5% glycerol	3	2	1
"B" + 4% glycerol	4	2	1
"B" + 3% glycerol	3	2	1
"B" + 2½% glycerol	3	2	2
"B" + 2% glycerol	3	2	2
"B" + 1% glycerol	2	1	1

*The arbitrary grading from 0 to 4 indicates the approximate amount of growth obtained from an average of 10 or more tubes seeded in each case. In the 1.0 column the readings recorded are after four weeks incubation, in the 0.000,1 mg. column after eight weeks, and in the 0.000,001 mg. column after ten weeks incubation.

c. *The Broth Concentration in the Medium.*—In order to test the effect of the broth concentration in the glycerol potato agar medium upon the growth of tubercle bacilli a basic medium ("M") was prepared containing 20 per cent ground fresh potato, 2 per cent pure glycerol, and 1.1 per cent agar-agar. To this was added varying amounts from 5 to 3 per cent of 4X beef broth, containing 12 gm. of Liebig's Beef Extract, 40 gm. of Bacto-peptone, and 20 gm. of pure sodium chloride in 1000 c.c. of tap water. The media after sterilization in the autoclave at 15 pounds for three minutes was

seeded with large and small amounts of virulent human tubercle bacilli and incubated for two months with the results recorded in Table IV.

The findings recorded in Table IV indicate that higher concentrations of broth added to glycerol potato agar mediums have a retarding influence upon the growth of the virulent human tubercle bacilli while in the presence of

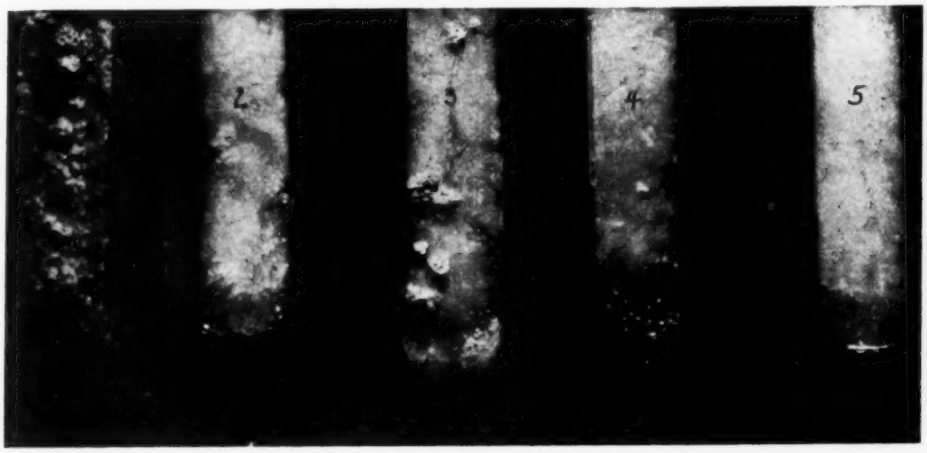


Fig. 1.—The effect of the individual components of glycerol broth potato cylinder medium upon the growth of virulent human tubercle bacilli eight weeks after seeding with 0,000, 1 mg. suspension.

Tube 1. Glycerol broth potato cylinder medium.
Tube 2. Broth potato cylinder medium without glycerol.
Tube 3. Potato cylinder medium with 5 per cent glycerol without broth.
Tube 4. Potato cylinder medium with 0.9 per cent sodium chloride solution; no glycerol or broth.
Tube 5. Potato cylinder medium without addition of liquids; no glycerol or broth.

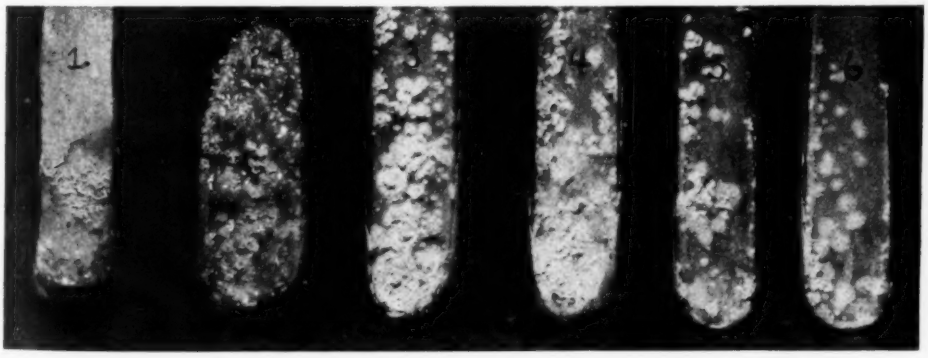


Fig. 2.—Growth of virulent human tubercle bacilli on medium containing 13.4 per cent 4X broth, 2 per cent glycerol, 1.1 per cent agar and varying amount of ground potato, six weeks after seeding with 0.000, 1 mg. suspension.

Tube 1. Glycerol broth potato cylinder medium.
Tube 2. Glycerol broth agar medium "A" with 60 per cent ground potato.
Tube 3. "A" with 30 per cent ground potato.
Tube 4. "A" with 20 per cent ground potato.
Tube 5. "A" with 10 per cent ground potato.
Tube 6. "A" with 5 per cent ground potato.

smaller amounts of from 5 to 10 per cent the growth occurred with the same vigor as upon the standard potato cylinder medium without crystal violet.

It might justifiedly be asked at this time whether the addition of broth

to the potato medium is necessary, since this was not touched upon in the foregoing studies. This will be given consideration later in the paper after we have presented the result of the study of optimum concentration of crystal violet capable of inhibiting the growth of the undesirable contaminators and still being innocuous to the growth and viability of the tubercle bacilli.

d. *The Crystal Violet Concentration in the Medium.*—In order to determine the efficiency of various concentrations of crystal violet for inhibiting

Fig. 3.—The growth of virulent human tubercle bacilli on broth agar potato medium containing 13.4 per cent 4X broth, 20 per cent ground potato, and 1.1 per cent agar with varying amounts of glycerol, three weeks after seeding with 1 mg. suspension.

Tube 1. Glyceral broth potato cylinder medium.
Tube 2. Broth agar potato medium "B" with 5 per cent glycerol.
Tube 3. "B" with 4 per cent glycerol.
Tube 4. "B" with 3 per cent glycerol.
Tube 5. "B" with 2 per cent glycerol.
Tube 6. "B" with 1 per cent glycerol.

Fig. 4.—The growth of virulent human tubercle bacilli on broth agar potato medium containing 13.4 per cent 4X broth, 20 per cent ground potato, and 1.1 per cent agar with varying amounts of glycerol, nine weeks after seeding with 0.000,001 mg. suspension.

Tube 1. Glycerol broth potato cylinder medium.
Tube 2. Broth agar potato medium "B" with 5 per cent glycerol.
Tube 3. "B" with 4 per cent glycerol.
Tube 4. "B" with 3 per cent glycerol.
Tube 5. "B" with 2 per cent glycerol.
Tube 6. "B" with 1 per cent glycerol.

the growth of the contaminators and permitting the unhampered growth of tubercle bacilli the basic medium ("M") was prepared containing 20 per cent ground fresh potato, 13.5 per cent 4X broth, 2 per cent glycerol, 1.1 per cent agar-agar, and 63.4 per cent tap water. To this medium was added varying amounts of standard crystal violet solution, using a 1 per cent alco-

holic solution for this purpose, so that the medium contained amounts of crystal violet ranging from 0.02 per cent to 0.001 per cent. In order to determine the effect of these mediums upon the growth of tubercle bacilli, they were seeded with suspensions of virulent human tubercle bacilli containing from 1.0 to 0.000,001 mg. of bacilli per cubic centimeter of suspension. In Table V are recorded the growth results from such seedings.

The results recorded in Table V indicate clearly that a slightly better growth occurs on the control glycerol potato broth medium without crystal

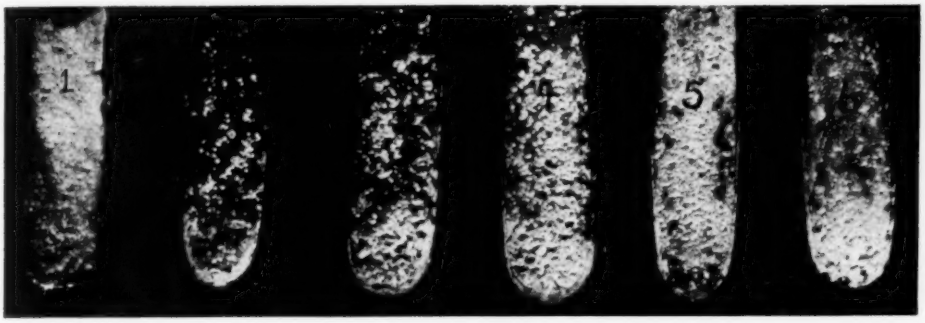


Fig. 5.—The growth of virulent human tubercle bacilli on glycerol agar potato medium containing 2 per cent glycerol, 20 per cent ground potato, 1.1 per cent agar and varying amounts of 4X normal strength of broth, four weeks after seeding with 1 mg. suspension.

Tube 1. Glycerol broth potato cylinder medium.
Tube 2. Glycerol agar ground potato "C" with 30 per cent 4X broth.
Tube 3. "C" with 20 per cent 4X broth.
Tube 4. "C" with 13.4 per cent 4X broth.
Tube 5. "C" with 10 per cent 4X broth.
Tube 6. "C" with 5 per cent 4X broth.

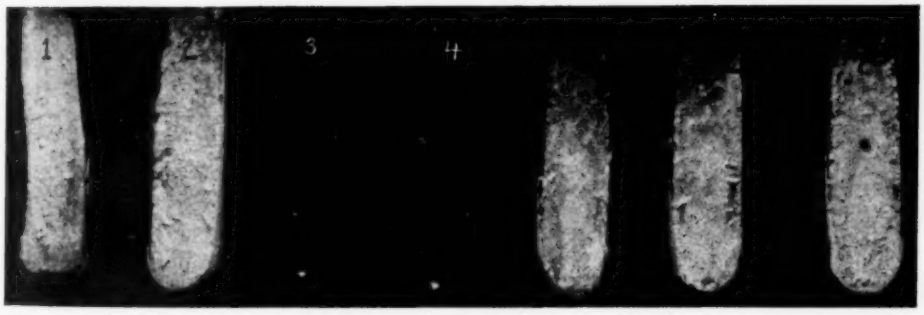


Fig. 6.—The growth of virulent human tubercle bacilli on glycerol agar broth potato medium containing 20 per cent potato, 13.4 per cent 4Xnormal strength broth, 2 per cent glycerol, 1.1 per cent agar with varying amounts of crystal violet, four weeks after seeding with 1 mg. suspension.

Tube 1. Crystal violet glycerol broth potato cylinder medium.
Tube 2. Glycerol agar broth potato medium "D" without crystal violet.
Tube 3. "D" with 0.02 per cent crystal violet.
Tube 4. "D" with 0.01 per cent crystal violet.
Tube 5. "D" with 0.002 per cent crystal violet.
Tube 6. "D" with 0.0015 per cent crystal violet.
Tube 7. "D" with 0.001 per cent crystal violet.

violet than it did on the control potato cylinder broth medium without crystal violet whether seeded heavily (1.0 mg.) or lightly (0.000,001 mg.), while all the glycerol potato broth mediums containing crystal violet in amounts down to 0.0015 per cent caused a retardation of growth.

For the purpose of testing out the value of these same crystal violet containing potato agar mediums in the isolation of tubercle bacilli from contaminated sources eight positive sputums were treated with an equal volume of 6 per cent sulphuric acid for thirty minutes at incubator temperature and after dilution and centrifugation the sediment was seeded on the mediums. The findings are recorded in summary form in percentages of isolation and contamination in Table VI.

TABLE IV

THE GROWTH OF VIRULENT HUMAN TUBERCLE BACILLI ON GLYCEROL POTATO AGAR MEDIUM CONTAINING VARYING AMOUNTS OF BEEF BROTH

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG. PER C.C. IN SUSPENSION USED FOR SEEDING CULTURE MEDIUM		
	1.0	0.000,1	0.000,001
Potato cylinder medium without crystal violet	3†	2	1
"M" (2% glycerol potato agar medium) + 30% 4X Broth*	1	1	0
"M" + 20% 4X Broth	2	1	1
"M" + 10% 4X Broth	3	2	1
"M" + 5% 4X Broth	3	2	1

*4X Broth indicates a concentrated broth mixture containing four times the usual amounts of solid constituents as are contained in a commonly used laboratory broth or 4X (3 gm. beef extract + 10 gm. peptone + 5 gm. sodium chloride) in 1000 c.c. tap water.

†The arbitrary grading from 0 to 4 indicates the approximate amount of growth obtained from an average of 10 or more tubes seeded in each case. In the 1.0 column the readings recorded are after four weeks incubation, in the 0.000,1 mg. column after eight weeks, and in the 0.000,001 mg. column after ten weeks incubation.

TABLE V

THE GROWTH OF VIRULENT HUMAN TUBERCLE BACILLI ON GLYCEROL POTATO BROTH AGAR CONTAINING VARYING AMOUNTS OF STANDARD CRYSTAL VIOLET

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG. PER C.C. IN SUSPENSION USED FOR SEEDING CULTURE MEDIUMS		
	1.0	0.000,1	0.000,001
Control: Potato cylinder medium without crystal viole.	3*	2	1
"M" (control glycerol potato broth agar without crystal violet)	4	3	2
"M" + 0.02% crystal violet	0	0	0
"M" + 0.01% crystal violet	0	0	0
"M" + 0.002% crystal violet	1	0	0
"M" + 0.0015% crystal violet	2	1	1
"M" + 0.001% crystal violet	4	2	1

*The arbitrary grading from 0 to 4 indicates the approximate amount of growth obtained from an average of 10 or more tubes seeded in each case. In the 1.0 column the readings recorded are after four weeks incubation, in the 0.000,1 mg. column after eight weeks, and in the 0.000,001 mg. column after ten weeks incubation.

The findings recorded in Table VI indicate that crystal violet incorporated in the glycerol potato broth agar medium result in a decreased percentage of tubercle bacillus isolations from the positive sputums as well as an absence of striking improvement in destroying contaminators in serviceable concentration of crystal violet as compared to the standard potato cylinder broth medium containing 0.0015 per cent crystal violet. Petroff's gentian violet egg medium proved superior to the agar mediums but inferior to the potato cylinder medium. There are probably two factors accountable for the inferior quality of the ground potato medium as compared to the cylinder medium; the

greater inhibitory influence of the crystal violet in the agar medium being one and the other possibly being due to the diminished value of the potato as a nutrient medium for the tubercle bacilli when oxidative changes have occurred coincident with a darkening of the freshly ground potatoes which was almost unavoidable in this type of preparation. The possibility that enzymatic (oxidase) oxidation destroyed the important active components of the potato for the growth of tubercle bacilli led to an experiment aiming to destroy the action of these enzymes. Therefore, before preparing the medium the whole unpeeled potato was first autoclaved at 15 pounds pressure for thirty minutes and was then peeled and ground and mixed with glycerol broth agar in the proportions of 30 per cent autoclaved ground potato, 13.5 per cent 4X broth, 2.5 per cent glycerol, 1.0 per cent agar, and 53 per cent tap water to form control medium "N" and to this was added varying proportions of crystal violet in concentrations from 0.01 to 0.0003 per cent. These mediums supported growth in the proportion revealed by the findings recorded in Table VII

TABLE VI

ISOLATIONS AND CONTAMINATIONS FROM SPUTUMS AFTER PRELIMINARY SULPHURIC ACID TREATMENT RESULTING ON GROUND POTATO BROTH AGAR MEDIUM CONTAINING VARYING AMOUNTS OF CRYSTAL VIOLET

CULTURE MEDIUM	CULTURE RESULTS* IN PERCENTAGE	
	POSITIVE ISOLATION OF TUBERCLE BACILLI	CONTAMINATIONS
Control: Potato cylinder broth medium + 0.0015% crystal violet	66	13
Petroff's gentian violet egg medium	47	13
"M" (control glycerol potato broth agar without crystal violet)	38	31
"M" + 0.01% crystal violet	0	3
"M" + 0.002% crystal violet	31	13
"M" + 0.001% crystal violet	34	19

*Five tubes of medium were seeded from each of the eight sputums on the different mediums tested.

TABLE VII

THE GROWTH OF VIRULENT HUMAN TUBERCLE BACILLI ON GROUND POTATO AGAR MEDIUM PREPARED FROM AUTOCLAVED WHOLE POTATOES AND CONTAINING VARYING AMOUNTS OF CRYSTAL VIOLET

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG. PER C.C. IN SUSPENSION USED FOR SEEDING CULTURE MEDIUMS		
	1.0	0.000,1	0.000,001
Control: Potato cylinder medium without crystal violet	3*	2	2
Control: Potato cylinder medium with 0.0015% crystal violet	3	2	2
"N" (Control: Autoclavated potato agar medium without crystal violet)	3	2	2
"N" + 0.01% crystal violet	0	0	0
"N" + 0.002% crystal violet	1	1	1
"N" + 0.001% crystal violet	2	2	2
"N" + 0.0003% crystal violet	3	3	3

*The arbitrary gradings from 0 to 3 for the amount of growth of the tubercle bacilli on the mediums are not identical to those recorded in the previous Tables I to V but are arbitrarily used to bring out finer differences in this tabulated study and therefore cannot be compared with the graded findings in the previous tables. The readings with the 1.0 mg. suspension seedings were made after four weeks incubation, with the 0.000, 1 mg. suspension seedings after eight weeks and with the 0.000,001 mg. after twelve weeks.

It is to be noted from the data presented in Table VII that the tubercle bacilli grew about equally well on the two potato cylinder mediums, with and without crystal violet, on the control medium "N" prepared from previously autoclaved whole potatoes, ground and mixed with glycerol broth and agar, and on the latter medium containing 0.000,3 per cent crystal violet. The same mediums were, therefore, used for the study of the isolation and contaminations developed from 8 positive tuberculous sputums after preliminary sulphuric acid treatment as in the previous experiment recorded in Table VI. These results are recorded in Table VIII.

It is to be noted from the data recorded in Table VIII that the medium prepared from autoclaved whole potatoes ground and mixed with glycerol broth and agar in suitable proportions and containing 0.000,3 per cent crystal violet yielded results in per cent isolations and contaminations about equal to that of potato cylinder broth medium prepared with 0.0015 per cent crystal violet. Greater amounts of dye in this medium were detrimental to growth for isolation purposes.

TABLE VIII

ISOLATIONS AND CONTAMINATIONS FROM SPUTUMS AFTER PRELIMINARY SULPHURIC ACID TREATMENT RESULTING ON POTATO BROTH AGAR MEDIUM, PREPARED FROM AUTOCLAVED POTATOES, CONTAINING VARYING AMOUNTS OF CRYSTAL VIOLET

CULTURE MEDIUM	CULTURE RESULTS IN PERCENTAGE	
	POSITIVE ISOLATIONS FOR TUBERCLE BACILLI	CONTAMINATIONS
Control: Petroff's gentian violet egg medium	69*	19
Control: Potato cylinder medium with 0.0015% crystal violet	88	13
"N" (Control: Autoclaved potato agar medium without crystal violet	75	28
"N" + 0.01% crystal violet	0	0
"N" + 0.002% crystal violet	66	3
"N" + 0.001% crystal violet	66	19
"N" + 0.0003% crystal violet	84	19

*Five culture tubes were seeded from each of the eight sputums on the different mediums tested.

e. *Growth of Tubercle Bacilli on Potato Without Broth.*—In the studies recorded in an earlier part of this paper it was noted that broth added to potato mediums in high concentrations had a deleterious influence upon the growth of the tubercle bacilli but that in low concentrations very little effect was noted upon their growth. This made it appear likely that the addition of broth might not be essential in the potato medium and this is borne out by the culture studies recorded in Table IX.

Since these preliminary experiments indicate that the addition of broth is not essential to obtaining good growth on potato mediums, we have initiated a more extensive study of this with pure suspensions of tubercle bacilli as well as trying isolations from positive sputums. This study has not advanced sufficiently far to report completely at this time but will be reserved for a subsequent complete contribution. However, Table X, records the tests with the potato cylinder and varying amounts of glycerol from 1 to 5 per cent in the 1.0 to 1.5 c.c. of tap water, appear sufficiently conclusive to record at this time.

It is thus to be noted from the data recorded in Table X that the bacilli grew about equally well especially in the high dilutions of seeding regardless whether broth was present in the solution at the bottom of the culture tubes into which the potato cylinders were dipped or whether sterile tap water containing 1 to 5 per cent glycerol was used for this purpose.

TABLE IX

THE GROWTH OF TUBERCLE BACILLI ON GROUND POTATO GLYCEROL AGAR MEDIUM IN THE ABSENCE OF BROTH

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG. PER C.C. IN SUSPENSION USED FOR SEEDING CULTURE MEDIUMS		
	1.0	0.000,1	0.000,001
Potato cylinder glycerol broth medium without crystal violet	3*	2	1
Ground potato glycerol† broth agar medium	4	2	1
Ground potato glycerol‡ agar medium without broth	4	2	1

*The arbitrary grading from 0 to 4 indicates the approximate amount of growth obtained from an average of 10 or more tubes seeded in each case. In the 1.0 column the readings recorded are after four weeks incubation, in the 0.000, 1 mg. column after eight weeks, and in the 0.000,001 mg. column after ten weeks incubation.

†This medium was prepared from autoclaved whole potatoes which were then mashed and 30 per cent added to 1.1 per cent agar-agar, 13.4 per cent four times normal strength broth, 2 per cent glycerol and 53.5 per cent tap water.

‡This medium was prepared from autoclaved whole potatoes which were mashed and 30 per cent added to 1.1 per cent agar-agar, 2 per cent glycerol and 66.9 per cent tap water but no broth.

TABLE X

THE GROWTH OF TUBERCLE BACILLI ON CRYSTAL VIOLET POTATO CYLINDERS DIPPED IN GLYCEROL SOLUTIONS WITHOUT BROTH

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG. PER C.C. IN SUSPENSION USED FOR SEEDING CULTURE MEDIUMS		
	1.0	0.000,1	0.000,001
Crystal violet potato cylinder in 1 c.c. 5% glycerol broth	4*	2	1
"P" (Crystal violet potato cylinder) in 1 c.c. 5% glycerol in tap water	3	2	1
"P" in 1 c.c. 4% glycerol water	3	2	1
"P" in 1 c.c. 3% glycerol water	3	2	1
"P" in 1 c.c. 2% glycerol water	3	2	1
"P" in 1 c.c. 1% glycerol water	3	2	1

*The arbitrary grading from 0 to 4 indicates the approximate amount of growth obtained from an average of 10 or more tubes seeded in each case. In the 1.0 column the readings recorded are after four weeks incubation, in the 0.000,1 mg. column after eight weeks, and in the 0.000,001 mg. column after ten weeks incubation.

The experiments in which the isolation of tubercle bacilli from contaminated pathologic specimens was studied using the glycerol tap water crystal violet potato cylinders without broth as culture medium has not progressed sufficiently far to warrant a definite recommendation of this medium, although the foregoing study with suspensions is encouraging.

Although the cooked ground potato crystal violet agar medium gave an efficiency about equal to the crystal violet potato cylinder broth medium, we prefer the crystal violet potato cylinder broth medium until a more exhaustive personal experience has disclosed no objections especially in uniformity of preparation of the cooked ground potato crystal violet agar medium. The details of preparation of this medium will, therefore, be reserved for a subsequent brief report at which time further experiences with the tap water glycerol crystal violet potato cylinder medium will be reported.

PART II

A Comparison of the New Sulphuric Acid Crystal Violet Potato Medium Method with Petroff's Sodium Hydroxide Gentian Violet Egg Medium Method and Guinea Pig Inoculation for the Isolation and Detection of Tubercle Bacilli in Sputums, Urines and Animal Tissues

In order to determine the comparative value of the new sulphuric acid crystal violet potato medium method, Petroff's sodium hydroxide gentian violet egg medium method and the sensitive guinea pig inoculation method for the isolation and detection of tubercle bacilli a series of microscopically positive and negative sputums, as well as urines, were used as test materials and in addition tissues (lung, liver, spleen, bone marrow, and kidney) from intravenously infected dogs and rabbits were submitted to analysis by these three methods.

a. *Detection of Tubercle Bacilli in Sputums.*—In order to compare the efficiency of the sulphuric acid crystal violet potato medium method with Petroff's[2, 3] sodium hydroxide gentian violet egg method on positive sputums, 93 microscopically positive sputums were used as tests and 366 culture tubes were seeded for each of the two methods. The summarized findings in this study are briefly recorded in Table XI. To have done guinea pig inoculation tests in this series would obviously have been unnecessary so they were omitted.

Table XI

A Comparison of the Sulphuric Acid Crystal Violet Potato Cylinder Method and Petroff's Sodium Hydroxide Gentian Violet Egg Method for the Isolation of Tubercle Bacilli From Sputums Microscopically Positive

Method of isolation used	Number of culture tubes of a total of 366 in which growth occurred	Percentage of total number of tubes in which growth occurred	Number of culture tubes of total 366 that were contaminated	Percentage of total tubes that were contaminated	Number of sputums of total 93 that were positive	Percentage of total sputums that were positive
Petroff's sodium hydroxide gentian violet egg medium method	129	35	27	7	41	44.0
Sulphuric acid crystal violet potato cylinder method	265	72	75	20	85	91.4

The findings with Petroff's method practically duplicate those previously recorded by other authors, and it is obvious, from the foregoing study and results recorded in Table XI, that the sulphuric acid crystal violet potato cylinder method gives a far greater number and percentage isolation from microscopic positive sputums.

In order to compare the efficiency of the two culture methods with guinea pig inoculation on sputums, a series of nine microscopically doubtful sputums from positive cases of pulmonary tuberculosis were used. Petroff's sodium

hydroxide gentian violet egg medium method gave growth in the case of two of these nine sputums, while the sulphuric acid crystal violet potato cylinder method and guinea pig inoculation gave positive findings in agreement in five of the total nine doubtful microscopic sputums. The remaining four sputums were negative by all the above three methods. It is thus to be noted that with doubtful sputums from definite cases, the sulphuric acid potato method was in entire accord with the results of guinea pig inoculation. There is, however, another important point worthy of mention at this time and that is that the guinea pig findings concurred with the sulphuric acid potato method in so far as the early appearance of positive findings only when the local glands were incised and smears examined for tubercle bacilli by staining methods, generalized disease with internal organ and splenic involvement in the guinea

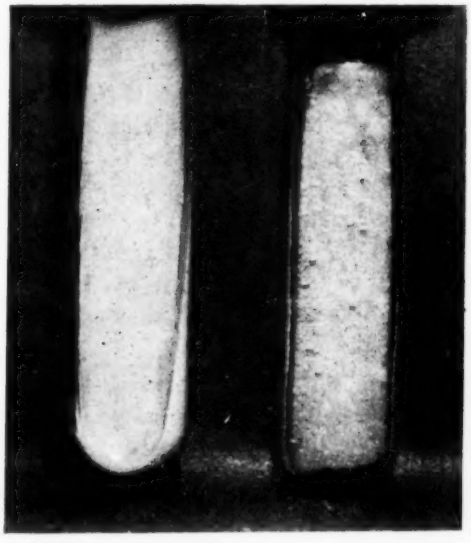


Fig. 7.—Isolation of tubercle bacilli from microscopically slightly positive sputum, after three weeks incubation, by the sodium hydroxide, gentian violet egg medium method (tube on left) and by the sulphuric acid, crystal violet potato cylinder method (tube on right). There was no growth from this sputum on the gentian violet egg medium even after ten weeks incubation, while growth appeared on the potato cylinder medium as early as the third week on all the tubes planted.

pig only develops several weeks later. Frequently smears from the glands are negative for tubercle bacilli in stained preparations and in many of these cases the sulphuric acid potato culture method yielded definite positive growth several weeks before the bacilli were found or definite generalized disease developed in the guinea pig. Petroff's method when positive gave a growth in practically all cases only several or more weeks later than positive evidence developed by the other two methods.

b. *Detection of Tubercle Bacilli in Urines.*—In order to compare the efficiency of Petroff's method, the sulphuric acid crystal violet potato cylinder method and guinea pig inoculation for detecting tubercle bacilli in urines, 24 specimens were collected and tested. Among these were positive and negative microscopic specimens. Of the 24 specimens of urine tested three gave posi-

tive cultures by Petroff's method with evident growth occurring only after the fifth week in one sample, and the tenth and thirteenth week respectively in the other two. The sulphuric acid crystal violet potato cylinder method gave evident growth in ten of the twenty-four specimens of urine while guinea pig inoculation gave positive findings in eleven of the twenty-four specimens. The one urine, however, which proved positive by guinea pig inoculation and not by the new culture method was positive only in one guinea pig while a duplicate remained entirely negative for tuberculosis even after three months' observation period. The failure of the culture method in this case may possibly have been due to the absence of tubercle bacilli from the inoculum used for seeding the culture tubes as was the case with the second guinea pig and this must therefore not necessarily be counted against the new culture method for detecting tubercle bacilli.

The earliest evidence for diagnostic purposes was possible with the new potato culture method, as evidenced by macroscopic growth of the tubercle bacilli on the medium, within the fourth or fifth week in the majority of the cases, while by the guinea pig inoculation method a diagnosis could be made in the third or fifth week provided the infected local glands were opened and a smear of the pus examined microscopically for tubercle bacilli. Generalized disease in the guinea pig with involvement of the spleen only occurred several weeks later. The above findings, therefore, indicate that the sulphuric acid crystal violet potato cylinder method is as efficient and as reliable as the guinea pig inoculation method for the detection of tubercle bacilli in sputums and urines.

c. *Detection of Tubercle Bacilli in Infected Tissues.*—The efficiency of the sulphuric acid crystal violet potato cylinder as compared to Petroff's method and guinea pig inoculation was also borne out in tests with infected animal tissues. For this part of the study 20 dogs and 20 rabbits were given intravenous injections of virulent human tubercle bacilli in varying graded amounts of 1.0, 0.01, 0.000,1, 0.000,001, and 0.000,000,01 mg. in fine suspension in sterile saline solution. These animals were then divided into two series, one being killed one week after infection and the second one month after infection. Tissues from five organs, e.g., lung, liver, spleen, kidney, and bone marrow, from each animal were examined. After being ground fine and mixed with sterile 0.9 per cent sodium chloride solution, approximately equal parts were used for the detection of tubercle bacilli by Petroff's method, the sulphuric acid crystal violet potato method and guinea pig inoculation. Specimen results of these studies are recorded in Tables XII, XIII, and XIV chosen at random from a large number of like test experiments with the tissues from rabbits and dogs but showing some significant findings.

The data obtained from the tissue studies in which dog's and rabbit's organs were used as tests and specimens of which are recorded in Tables XII, XIII, and XIV revealed that in general most of the results of guinea pig inoculation were in accord with the findings in the sulphuric acid potato cylinder culture method. At times, however, when the bacilli were sparse in the tissues, due to the small amount given intravenously to the animals,

the sulphuric acid potato method would give positive findings while the guinea pig inoculation method was negative, and vice versa.

Since in earlier studies a comparison was made between the crystal violet potato cylinder broth medium and Petroff's gentian violet egg medium which proved the former medium to be far superior for promoting the growth of small numbers of tubercle bacilli seeded thereon than the latter it was also deemed advisable to perform comparative tests of the sodium hydroxide used in Petroff's method for the preliminary treatment of tuberculosis materials and the sulphuric acid used in the sulphuric acid potato method. In addition

TABLE XII

A COMPARISON OF GUINEA PIG INOCULATION AND THE H_2SO_4—POTATO CULTURE METHOD WITH PETROFF'S METHOD FOR THE DETECTION OF TUBERCLE BACILLI IN RABBIT TISSUES

TISSUES FROM RABBIT INFECTED INTRAV. (1 MONTH) WITH 1.0 MG. VIR. HUMAN TUBERCLE BACILLI	PETROFF'S METHOD								NEW METHOD								GUINEA PIG INOCULATION									
	GROWTH EVIDENCE CONCLUSIVE								GROWTH EVIDENCE CONCLUSIVE								ANTEMORTEM DIAGNOSIS (EVIDENCE NOT CONCLUSIVE)*								POSTMORTEM DIAGNOSIS (EVIDENCE CONCLUSIVE)	
	NO. OF WEEKS AFTER INOCULATION								NO. OF WEEKS AFTER INOCULATION								NO. OF WEEKS AFTER INOCULATION								NO. OF WEEKS AFTER INOCULATION	
	2	3	4	5	6	7	8	14	2	3	4	5	6	7	8	14	2	3	4	5	6	7	8	16	6	16
Bone marrow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?	+	+	+	+		+
Kidney	0	0	0	0	0	0	0	+	0	?	+	+	+	+	+	+	0	0	+	+	+				+	
Liver	0	0	0	0	0	0	0	0	0	0	?	+	+	+	+	+	0	?	?	?	?	?	?	+		+
Lung	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	?	?	+	+					+(?)†	
Spleen	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	0	0	?	?	+	+	+	+		+

*By antemortem diagnosis is designated the local gland involvement following subcutaneous injection. When the enlargement of the local glands warranted, they were incised and examined for the presence of tubercle bacilli and when present the animal was sacrificed within a week or two and examined for generalized disease which is then recorded under Postmortem Diagnosis. Only three gradings are used in these tabulations O, negative; ?, the diagnosis is uncertain or glandular enlargement was insufficient to make a definite diagnosis; and +, the presence of definite growth of bacilli, definite glandular involvement or generalized tuberculosis dependent upon the column under which recorded.

†In this guinea pig most of the glands (inguinal, retroperitoneal, and tracheal) were enlarged but no bacilli were found in smears and the spleen and rest of the organs were not tuberculous.

TABLE XIII

A COMPARISON OF GUINEA PIG INOCULATION AND THE H_2SO_4—POTATO CULTURE METHOD WITH PETROFF'S METHOD FOR THE DETECTION OF TUBERCLE BACILLI IN RABBIT TISSUES

TISSUES FROM RABBIT INFECTED INTRAV. (1 MONTH) WITH 0.01 MG. VIR. HUMAN TUBERCLE BACILLI	PETROFF'S METHOD								NEW METHOD								GUINEA PIG INOCULATION									
	GROWTH EVIDENCE CONCLUSIVE								GROWTH EVIDENCE CONCLUSIVE								ANTEMORTEM DIAGNOSIS (EVIDENCE NOT CONCLUSIVE)*								POSTMORTEM DIAGNOSIS (EVIDENCE CONCLUSIVE)	
	NO. OF WEEKS								NO. OF WEEKS								NO. OF WEEKS								NO. OF WEEKS	
	2	3	4	5	6	7	8	14	2	3	4	5	6	7	8	14	2	3	4	5	6	7	8	16	6	16
Bone marrow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0
Kidney	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0
Liver	0	0	0	0	0	0	+	+	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0		0
Lung	0	0	0	0	0	0	+	+	0	0	0	+	+	+	+	+	0	0	+	+	+				+	
Spleen	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	?	?	+	+	+	+	+	+		+

*By antemortem diagnosis is designated the local gland involvement following subcutaneous injection. When the enlargement of the local glands warranted, they were incised and examined for the presence of tubercle bacilli and when present the animal was sacrificed within a week or two and examined for generalized disease which is then recorded under Postmortem Diagnosis. Only three gradings are used in these tabulations O, negative; ?, the diagnosis is uncertain or glandular enlargement was insufficient to make a definite diagnosis; and +, the presence of definite growth of the bacilli, definite glandular involvement or generalized tuberculosis dependent upon the column under which recorded.

it was considered advisable to determine whether a residence on Petroff's egg medium as well as the crystal violet potato cylinder may not exert a detrimental influence upon the bacilli from tuberculous tissues planted on these mediums. The results of these tests using the guinea pig as test animal for determining the viability of the bacilli is recorded in Table XV.

It is clearly evident from the data recorded in Table XV that both the sodium hydroxide treatment and the culturing of the prepared plant on the gentian violet egg medium as prescribed for Petroff's method have a slight but definite detrimental influence upon the viability of small numbers of tubercle bacilli contained in both the spleen of the rabbit and the liver of the dog as determined by guinea pig inoculation, which injurious effect upon

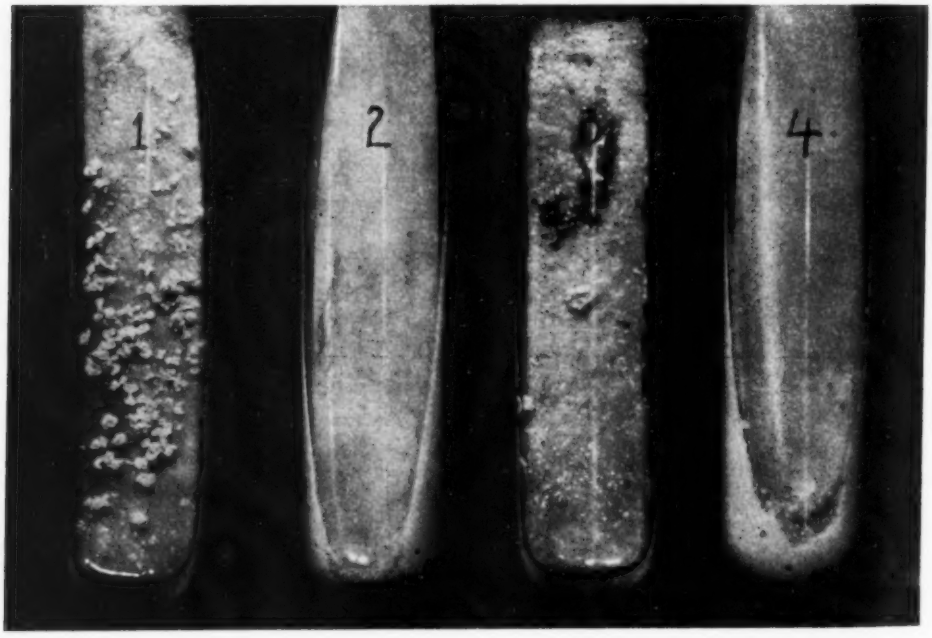


Fig. 8.—A comparison of cultures from the spleen of rabbits, intravenously infected with virulent human tubercle bacilli, eight weeks after isolation by Petroff's method (Tubes 2 and 4) and by the sulphuric acid, crystal violet potato cylinder method (Tubes 1 and 3). The isolation in Tubes 1 and 2 was from a rabbit given an intravenous injection of 1 mg. of bacilli and in Tubes 3 and 4, 0.01 mg. Tubes 1 and 3 revealed growth as early as the third week while Tubes 2 and 4 were still negative after twelve weeks incubation.

the bacilli was not evident after the sulphuric acid treatment and seeding upon the crystal violet potato cylinder broth medium. These findings account for the greater delicacy of the sulphuric acid potato method as compared to the sodium hydroxide gentian violet egg method as well as for the higher percentage isolations from sputums, urines, and animal tissues obtained with the former method. These data, recorded in Table XV, also again emphasize the efficacy of the sulphuric acid potato method for isolating tubercle bacilli from materials infected with tubercle bacilli and its equality in percentage isolations to the guinea pig over which it has many advantages especially in convenience of examination and maintenance.

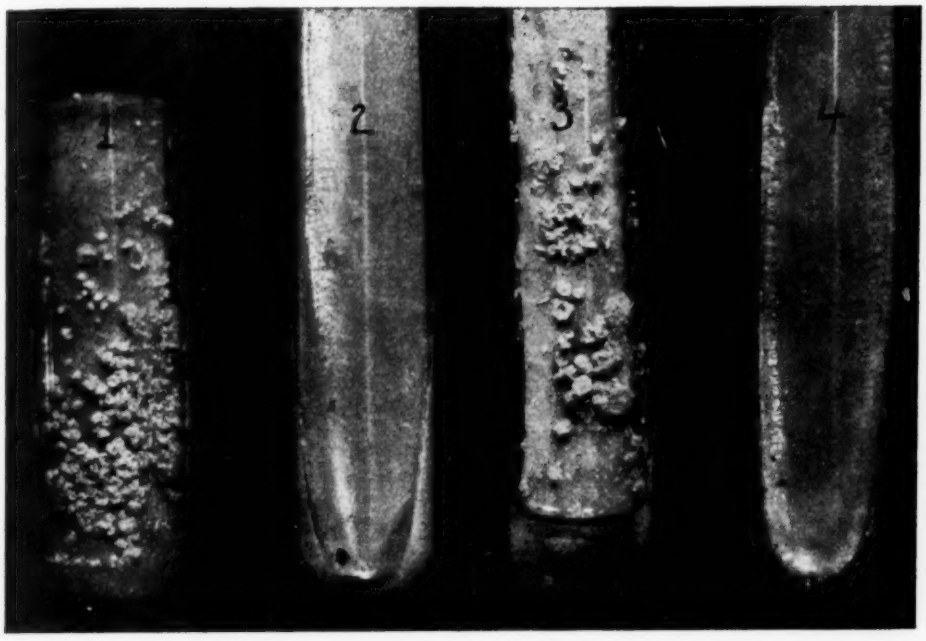


Fig. 9.—A comparison of cultures from the liver of dogs intravenously infected with virulent human tubercle bacilli, eight weeks after isolation by Petroff's method (Tubes 2 and 4) and by the sulphuric acid, crystal violet potato cylinder method (Tubes 1 and 3). The isolation in Tubes 1 and 2 was from a dog given an intravenous injection of 1 mg. of bacilli and in Tubes 3 and 4, 0.01 mg. Tubes 1 and 3 revealed growth as early as the third week while Tube 2 revealed a sparse growth at eight weeks as shown above and Tube 4 was negative to the twelfth week.

TABLE XIV

A COMPARISON OF GUINEA PIG INOCULATION AND THE H_2SO_4—POTATO CULTURE METHOD WITH PETROFF'S METHOD FOR THE DETECTION OF TUBERCLE BACILLI IN DOG TISSUES

TISSUES FROM DOG INFECTED INTRAV. (1 MONTH) WITH 0.000,1 MG. VIR. HUMAN TUBERCLE BACILLI	PETROFF'S METHOD								NEW METHOD								GUINEA PIG INOCULATION									
	GROWTH EVIDENCE CONCLUSIVE								GROWTH EVIDENCE CONCLUSIVE								ANTEMORTEM DIAGNOSIS (EVIDENCE NOT CONCLUSIVE)*								POSTMORTEM DIAGNOSIS (EVIDENCE CONCLUSIVE)	
	NO. OF WEEKS								NO. OF WEEKS								NO. OF WEEKS								NO. OF WEEKS	
	2	3	4	5	6	7	8	14	2	3	4	5	6	7	8	14	2	3	4	5	6	7	8	16	6	16
Bone marrow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0
Kidney	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0
Liver	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	0	0	?	?	?	(Died)			0	
Lung	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	?	+	+	+	+	+	+	+		+
Spleen	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	0	0	0	0	0	0	0	0		+†

*By antemortem diagnosis is designed the local gland involvement following subcutaneous injection. When the enlargement of the local glands warranted, they were incised and examined for the presence of tubercle bacilli and when present the animal was sacrificed within a week or two and examined for generalized disease which is then recorded under Postmortem Diagnosis. Only three gradings are used in these tabulations O, negative; ?, the diagnosis is uncertain or glandular enlargement was insufficient to make a definite diagnosis; and +, the presence of definite growth, definite glandular involvement or generalized tuberculosis dependent upon the column under which recorded.

†Although this animal revealed a slight nodular lesion in the abdominal wall after the eighth to tenth week there developed no enlargement of the local glands and at postmortem after four months there was found only a slight splenic involvement with several small tubercles but no enlargement of this organ and no involvement of the other organs. The retroperitoneal and tracheal glands were also slightly enlarged but not significantly so.

TABLE XV

THE EFFECT OF THE PRELIMINARY TREATMENT WITH SODIUM HYDROXIDE OR SULPHURIC ACID, AND PLANTING ON GENTIAN VIOLET EGG MEDIUM OR CRYSTAL VIOLET POTATO MEDIUM ON THE VIABILITY OF TUBERCLE BACILLI CONTAINED IN TISSUES

EXPERIMENT	TISSUE TESTED																			
	SPLEEN OF RABBIT GIVEN INTRAVENOUS INJECTION 1 WEEK BEFORE EXAMINATION										LIVER OF DOG GIVEN INTRAVENOUS INJECTION 1 WEEK BEFORE EXAMINATION									
	0.01 MG.					1.0 MG.					0.01 MG.					1.0 MG.				
	INTERVAL IN WEEKS FOLLOWING SEEDING OF TUBES OR INJECTION OF GUINEA PIG																			
	–	4	6	8	12	2	4	6	8	12	2	4	6	8	12	2	4	6	8	12
Control guinea pig inoculated with tissue direct	0*	±	+	+	+2	+	++	+++ 3			0	+	++ 2			+	++	+++ 4		
Guinea pig inoculated with tissue treated with H_2SO_4 alone	0	+	++ 2			±	++	+++ 3			±	+	++ 2			+	++	++ 3		
Guinea pig inoculated with tissue treated with H_2SO_4 and incubated for 3 weeks on potato medium	++ u	++ u	+ u	+ u 4		++ u	++ u	++ u	+ u 4		+ u	++ u	+ u	+ u 4		++ u	++ u	++ u	++ u 4	
Culture results after H_2SO_4 treatment and planting on potato cylinder medium	0	1†	2	3	4	0	2	3	4	4	±	1	2	4	4	1	2	4	4	4
Guinea pig inoculation with tissue treated with NaOH (Petroff's alone)	0	+	+	+	+ 1	±	+	+ 1			0	+	+ 2			±	+	++ 2		
Guinea pig inoculated with tissue treated with NaOH and incubated for 3 weeks on Petroff's egg medium	0	0	0	0	0 0	0	±	+	++ 1		0	0	0	0	0 0	+	++	++	++ 3	
Culture results after NaOH treatment and planting on Petroff's egg medium	0	0	0	0	0	0	0	0	0	0	0	0	0	1‡	1	0	0	0	1	2§

*The infected tissues from the dogs and rabbits were used one week after intravenous injection of a strain of virulent human tubercle bacilli. The tuberculous involvement of the local site of subcutaneous inoculation and local glands is graded from 0 to ++++ according to the grade of palpable enlargement. The numeral accompanying the last grading recorded indicates that at postmortem after the number of weeks under which column the figure is given the animal presented either 0, no tuberculous involvement; 1, enlargement of the local glands alone; 2, enlargement of the local and tributary glands and slight involvement of the spleen; 3, generalized glandular enlargement, marked splenic involvement, and enlargement and slight hepatic and pulmonary involvement; and 4, a marked generalized involvement of most organs with marked splenic enlargement and involvement. A "u" after the local gland grading indicates that the local lesion has ulcerated and is discharging. The guinea pig readings recorded in each case are the average for two animals inoculated for each tissue tested or for separate animals inoculated from two separate culture tubes if the bacilli had been seeded on these before inoculation.

†The reading recorded under the culture results is the average of at least two culture tubes seeded and usually of 3 or 4 culture tubes. The amount of growth is graded from 0, no macroscopic evident growth to 4, a profuse thick growth covering most of the medium.

‡Growth was obtained in this case on only one of three culture tubes.

§Growth was obtained in this case on two of three culture tubes.

SUMMARY AND CONCLUSIONS

For the isolation of tubercle bacilli from contaminated tuberculous materials, such as urines, sputums, and tissues, the sulphuric acid crystal violet potato cylinder method is superior to the sodium hydroxide gentian violet egg medium method and equal in efficiency to the guinea pig inoculation test. Small numbers of tubercle bacilli in tissues (dog's liver and rabbit's spleen) are not appreciably affected by the preliminary treatment with an equal volume of 6 per cent sulphuric acid at 37° C. for thirty minutes used to destroy the contaminators nor by planting on the crystal violet potato cylinder medium while an equal volume of 3 per cent sodium hydroxide for twenty minutes at 37° C. has a slight detrimental influence upon the tubercle bacilli which is further enhanced by seeding on gentian violet egg medium as prescribed in Petroff's method.

Ground fresh potatoes suffer changes during grinding and exposure to air which makes them less suitable in the preparation of glycerol agar mediums for growing tubercle bacilli than like mediums made from potatoes ground and mixed with the medium after having been autoclaved for thirty minutes at 15 pounds pressure. In the preparation of the crystal violet potato cylinder medium the amount of glycerol, from 1 per cent to 5 per cent, in the broth or tap water used, has no appreciable influence upon the growth of the tubercle bacilli on this medium. It appears from preliminary studies that tap water containing the glycerol can be substituted for the glycerol broth in the preparation of the medium. Likewise it appears that a glycerol crystal violet potato agar medium in which the potatoes were autoclaved before being ground may be substituted for the crystal violet potato cylinder broth medium used for isolating tubercle bacilli. This is being more exhaustively studied. The glycerol content in this medium, however, requires to be about 2 to 2½ per cent. If broth is used in the preparation of this medium it must not be used in excess. The most favorable concentration of crystal violet added to this medium neutralized with sodium carbonate solution is about 0.000,3 per cent, whereas with the potato cylinder medium the cylinders are best dipped in 0.0015 per cent dye containing 1 per cent sodium carbonate.

The sulphuric acid crystal violet potato method for isolating and detecting tubercle bacilli possesses many advantages over guinea pig inoculation including the possibility of repeated observations, economy of performance, availablility of a culture, when positive, for determining the type of bacilli present as well as adding a feeling of confidence and certitude in the findings.

REFERENCES

1. Corper, H. J., and Uyei, Nao: The Isolation of Tubercle Bacilli From Contaminated Tuberculous Material, Am. Rev. Tuberc. 16: 299-322, 1927; ibid. 9: 115-130, 1927. The Cultivation of Tubercle Bacilli. An Improved Method for Isolation From Tuberculous Materials, J. Lab. & Clin. Med. 13: 469-480, 1928.

2. Petroff, S. A.: A New and Rapid Method for the Isolation and Cultivation of Tubercle Bacilli Directly From the Sputum and Feces, J. Exper. Med. 21: 38-42, 1915. Some Cultural Studies on Tubercle Bacilli, Bull. Johns Hopkins Hosp. 26: 276-279, 1915.

3. Corper, H. J., Fiala, L., and Kallen, L.: The Routine Cultivation of Tubercle Bacilli From the Sputum by Petroff's Method, J. Infect. Dis. 23: 267-274, 1918.

‡Growth was obtained in this case on only one of three culture tubes.

DISCUSSION

Dr. Henry C. Sweany.—I am much gratified to hear of this work that Dr. Corper has been doing. I must say that we have been working along the same line and just in a few words I should like to state some of our findings. We also soon found out that the Petroff method fails to give the best results. In a series of sinuses and glands and various other pathologic specimens from our institution we obtained a 4 per cent growth and by a medium that we have been working on ourselves, we obtained 47 per cent growth. There is no question about the inadequacy of the Petroff method for fine results. As I was coming down this morning I heard some one state that one of his medical instructors told him that 85 per cent of bacilli from the sputum were dead. I should hate to have one per cent injected into me. Where you have the proper culture medium and where you use a proper technic you can get practically all of them to grow. They are all virulent. We use an egg media, making it with a great deal of care. We substitute milk in place of the water, and cream instead of the glycerol. Bovine bacilli grow well on this medium even fresh from bovine lesions. We have felt that the greatest drawback to the Petroff's method was due to the gentian violet, and we have improved it by adding milk and cream. Our results are comparable with Dr. Corper's.

Dr. A. H. Sanford.—I should like to ask Dr. Corper a few questions. I have asked this question before. What about the culture of the tubercle bacilli in the urine especially where you have a small quantity of urine and you want to get the most from the material and are unable to demonstrate any acid-fast bacilli. Just what technical procedure would you follow to be sure that you could substitute your culture method for guinea pig inoculation. I should like to ask if you have made any study at all of "Much's Granules."

Dr. H. J. Corper (closing).—Time did not permit reading the entire paper which presents many additional details and will answer many questions regarding the new method. The sulphuric acid potato method is used with urine, sputum, and tissues. The potato method has proved to be speedier of performance, more satisfactory when reading results and equal in efficiency to the guinea pig. With your permission I will read a paragraph on urine from our paper. (See manuscript, "*Detection of Tubercle Bacilli in Urine.*") In some cases of genitourinary tuberculosis tubercle bacilli can be found in the centrifugated sediment by microscopic examination, but in the absence of a positive microscopic finding we resort to culture which has then proved positive in many additional specimens.

Errors can be obviated by using a simplified technic and with reasonable precautions such as avoiding dirty corks or test tubes. The use of new slides is advised because tubercle bacilli may stick to slides and cause confusion in findings. In the new culture method we have stressed the simplification of technic. Potato and glycerin with a small amount of crystal violet composes the medium; sulphuric acid and the medium completes the essentials to the method.

In reply to the question regarding Much's granules I might remind you that only acid-fast bacilli are of diagnostic value. Spengler described bodies, coccoid in type, which were acid fast, many years ago and they were no doubt the remnants of tubercle bacilli in the course of destruction but they have proved of little value in diagnosis. Much's method is worthless. The method is nothing but a Gram method and when you realize that there is gram-positive material in many bacteria in no way related to tubercle bacilli you will not depend upon such an index for diagnostic purposes. A great many tissues and tissue débris will stain by the Gram method. It is not peculiar that tubercle bacilli should contain some of these chemical substances staining by Gram. The tubercle bacilli contain metachromatic granules and in addition they possess polar bodies which reduce silver after impregnation. Carbol-fuchsin stains all these elements and differentially so that special Gram staining or silver impregnation is redundant for practical purposes.

So far as we are able to interpret at present the acid-fast forms of tubercle bacilli still stand as the forms that concern us in disease production. I am of the opinion that tubercle bacilli are destroyed in the body and as lysis progresses the acid-fast form disappears but I believe only after propagation and the disease producing character of the organism is likewise lost. Until proved otherwise for practical purposes it is best that we only accept the acid-fast forms as of diagnostic significance.

TUBERCULOUS ENDOMETRITIS*

By Harry L. Reinhart, B.A., M.D., and Robert A. Moore, M.Sc., M.D.
Columbus, Ohio

ACCORDING to most textbooks, and authors who have reviewed it, tuberculosis of the female genital tract is of rather common occurrence. Yet a review of the literature on the subject reveals very few specific cases and a rather scant discussion of the subject in general. While this does not justify the conclusion that female genital tuberculosis is rare, from the number of cases we have encountered in our own laboratories, we are of the opinion that it is not as common as is often assumed.

Statistics vary considerably, but none indicate any great frequency in the general run of clinical material. Thus Frank[1] cites Fromme and Heynemann who in 17,470 autopsies found genital tuberculosis in 142, or about 0.81 per cent. Schlimpert,[2] in 3514 autopsies of women and children *with tuberculosis,* found 3.4 per cent affected with genital lesions. Naturally the incidence of infection must be greater in cases of active tuberculosis than in the general run of clinical and autopsy material, and when we consider that the tubes are most frequently infected, we see at once that tuberculosis of the uterus is not common.

The assumption that primary tuberculous infection occurs in the female genital tract has always been very attractive, especially in view of the manifest portal of entry. But definite evidence of such a mode of infection has never been produced. Such evidence as has been brought forward in support of this theory has been purely circumstantial. Thus Bauereisen[3] cites an autopsy upon the widow of a phthisical man in which tuberculous endometritis was the only lesion of tuberculosis found. The primary lesion of tuberculosis is not necessarily a macroscopic lesion, and the bacilli may gain access to the body and be widely disseminated without any definite macroscopic evidence of the original portal of infection. One should not assert that primary infection of the female genital tract may occur, upon circumstantial evidence.

Many lesions of tuberculosis that were formerly considered primary lesions, in the light of more recent experimental investigations, appear to be secondary lesions. The pathology of a primary focus of infection is different from that of a secondary, whether this infection be endogenous or exogenous. The primary focus is small, discrete, sharply defined, composed almost exclusively of epithelioid cells, and usually shows coexistent involvement of the regional lymph nodes, whereas the secondary infection is greater in area, less sharply defined, variable in component elements and does not show the same tendency to heal that is manifest in the primary focus.

*Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minnesota, June 8, 9, and 11, 1928.

From the Department of Pathology, The Ohio State University, Columbus, Ohio.

If most tuberculous infections are secondary, the question arises as to the mode of infection of the female genital tract. Direct extension from an adjacent lesion such as a tuberculous peritonitis is possible, but it is probable that with a coexistence of these two lesions the original extension was from the female genital tract to the peritoneum rather than in the opposite direction since the concomitant peritonitis is frequently localized in the pelvis, and intestines and mesenteric lymph nodes are often unaffected. Infection in the majority of cases is metastatic and by the blood stream and the original focus is most frequently in the lungs or bronchial lymph nodes. Such an original focus also bespeaks the infrequency of lymphatic metastasis to the female genital tract. Metastatic extensions in cases of manifest pulmonary tuberculosis are far more widely disseminated than macroscopic examination reveals. Such disseminated foci are only revealed by extensive and careful microscopic examination of all organs. Even then the lesions do not always exhibit the typical textbook form, and special staining of the tubercle bacilli may be required to demonstrate their nature. This indicates that dissemination of tubercle bacilli are frequent and that the development of a macroscopic lesion in organs is rare in proportion to the number of individuals who are infected with tuberculosis, and are manifested in miliary tuberculosis or isolated tuberculosis of some organ or system. Some organs are affected more frequently than others; this has led to the assumption that there is a difference in the resistance of organs to tuberculous infection. There is a difference in resistance but probably not so much an inherent difference in the soil as in the physiologic activities of the particular organ. Thus Krause (quoted by Baldwin, Petroff and Gardner[4]) has suggested that in organs constantly in motion the lymph flow is so active that the bacilli are not allowed to remain in one place sufficiently long for the development of a lesion. In the uterus we not only have an organ which is contracting periodically, but a congestion and desquamation of the entire endometrium, which practically amounts to a physiologic excision of the tuberculous area. Cases of tuberculous endometritis cured by curettage have been reported. The normal endometrium performs a physiologic curettage periodically. We know this prevents the establishment of chronic foci of infection in the endometrium with the common bacteria, and we believe it to be a factor in the prevention of the establishment of chronic tuberculous foci. If this be true, we should expect to find evidence of some primary process that might alter this function along with tuberculous infections, and such is true both in our own cases and in those reported by others. The frequency of tuberculosis in the placenta, following multiple pregnancies, in association with leiomyomas, fibrosis uteri, and even carcinoma, supports this view. Thus tuberculosis is a secondary and complicating lesion in these conditions and not a primary lesion as is usually assumed.

The macroscopic appearance of tuberculous tubes and uteri in 75 per cent of the cases cannot be distinguished from those due to other infecting organisms. A varying number of cases associated with a general tuberculous peritonitis, do not represent true genital tuberculosis, but are a part of another process. In these cases the sago-like tubercles on the surface of the tube and mesosalpinx are really a part of a peritonitis and frequently there is no evidence

of tuberculosis in the genital tract itself. The true genital tuberculosis with which we are concerned is the type with definite lesions in the mucosa or muscularis of the tubes or uterus and in this type there is frequently no characteristic to identify the process on gross examination, and it is these cases that are frequently assigned to some other etiologic agent, especially when routine microscopic examination is not made of every tube or uterus that is removed.

Clinically there is no pathognomonic symptom or even suggestive symptom associated with tuberculous infection of the female genital tract. In the tubes the infection is most frequently assigned to gonococcus or puerperal infection, according to the history. In the uterus, a diagnosis of malignancy, adenomyoma, fibromyoma or gonococcus infection is usually made, according to the history and symptoms. In the cervix the differentiation between malignancy and tuberculosis most frequently leans toward malignancy, and indeed the differentiation is difficult with the gross specimen and may give rise to no little concern in the microscopic section.

The age of occurrence is of interest in this connection. White[5] found approximately 80 per cent occurring between the ages of sixteen and thirty-six. In general it may be said that pelvic infection of obscure cause and with atypical symptoms between sixteen and thirty-six years of age is always suggestive of tuberculosis. Clinical search for a primary focus of tuberculosis often reveals none. Curettement of the uterus and microscopic examination may yield tubercles, or may be so atypical that the diagnosis is confusing. The infrequency of tuberculous myometritis, and when it does occur the frequent association with other pathologic lesions, especially adenomyoma or fibroma, suggests that a primary pathologic alteration of the physiology of the body of the uterus is a factor in tuberculous infection. Such alterations most manifestly disturb the muscular physiology, and thus the lymphatic drainage.

CASE REPORT

L. R., aged thirty, Africo-American, was admitted to the hospital with a complaint of "pain in the left side." She was married at the age of thirteen years, had had 3 normal pregnancies, in 1910, 1912, and 1914 respectively. The onset of pain was approximately twelve years ago, in 1916, about two years after her last pregnancy. She was eighteen years of age at that time. The only other symptom was a marked leucorrhea. Catamenia was regular but profuse. The physical examination was negative with the exception of definite tenderness in the left lower quadrant. The blood count was not significant and the Wassermann was negative. The x-ray examination of the chest was negative. Pelvic examination was negative except for a rather definite tenderness of the left adnexa. A tentative diagnosis of gonorrheal salpingitis was made and a panhysterectomy performed. The uterus was enlarged and boggy and contained subserous and intramural fibroids. The right tube was bound to the ovary, forming a tubo-ovarian mass. Extensive omental adhesions bound the pelvic viscera in a mass. There was no evidence of tubercles on the serous surface of either ovaries, tubes, or the uterus. The endometrium was polypoid.

Microscopic examination revealed numerous tubercles scattered throughout the endometrium and tubal mucosa and the ovaries. The tubercles were of the giant cell type, with many epithelioid cells. There was little tendency toward caseation, coalescence, or conglomerate tubercle formation, and the size of the tubercles was uniform. Complete encapsulation was lacking, although the process did not appear to be very active. The tubercles were scattered throughout the endometrium, and pathologically they appeared to be of recent origin. The associated fibromyomas and fibrosis uteri were significant. There was no invasion of the myometrium.

This case appeared to be one of subinvolution, with multiple fibroids, and a resulting polypoid endometrium upon which subsequently appeared a tuberculous infection. The tuberculous process was certainly not of twelve years duration, which was the period over which the patient had exhibited symptoms. The tuberculous process appeared to be of rather recent origin, while the subinvolution and fibroids were the older process, and were undoubtedly the cause of her earlier symptoms. No evidence of a primary focus was found clinically, which of course does not eliminate such a focus. We believe the infection of both tubes and endometrium was metastatic, probably from a focus somewhere in the respiratory tract.

A second case presented a uterus that was slightly enlarged, and markedly fibrosed. On cut section the body of the uterus was dotted with small definite discrete yellow caseous nodules, about 1 mm. in diameter. There were none in the endometrium which was compressed by the thickening of the wall of the uterus. Microscopic examination revealed a marked fibrosis uteri; the nodules were caseous but definitely encapsulated by the fibrosis of the uterus. There were also small tubercles present of the giant cell type, with many epithelioid cells without encapsulation or caseation. The endometrium was atrophic and many microscopic sections revealed only one definite tubercle at the junction of endometrium and myometrium. The tubercles in the myometrium were at varying ages, some being early and others old, large, and caseous.

The sequence in this case seems to have been a subinvolution, fibrosis uteri, and tuberculous infection, with extension of the process to other parts of the uterus.

SUMMARY

1. There is a marked variation in the reported frequency of tuberculous lesions in the female genital tract.

2. The physiologic activities of various portions of the female genital tract may be responsible for variations in resistance to tuberculous infection.

3. Tuberculosis of the uterus is more frequently superimposed upon a pathologic than a normal organ.

REFERENCES

1. Frank, R. T.: Gynecological and Obstetrical Pathology, New York, D. Appleton and Co., pp. 536, 1922.
2. Schlimpert, H.: Die Tuberkulose bei der Frau etc., Arch. f. Gynäk. 94: 863-925, 1911.
3. Bauereisen, A.: Experimenteller Beitrag zur Kohabitationstuberkulose der Weiblichen Genitalien, Zentralbl. f. Gynäk. 44: 905-914, 1920.
4. Baldwin, E. R., Petroff, S. A., and Gardner, L. S.: Tuberculosis: Bacteriology, Pathology and Laboratory Diagnosis, Philadelphia, Lea and Febiger, pp. 342, 1927.
5. White, C.: Eden and Lockyer's New System of Gynecology, London, Macmillan & Co. 1: 611, 1917.

THE CHEMISTRY AND CYTOLOGY OF SEROUS FLUIDS*

By Alvin G. Foord, M.D., Guy E. Youngberg, Ph.D., and Vera Wetmore, B.S.,
Buffalo, N. Y.

VERY little information regarding the amounts of various chemical constituents in serous effusions is found in reviewing recent literature since the advent of modern methods of blood and serum chemistry. Most of the articles available give data on only a few of the chemical substances present. No report on a complete study of a large series of fluids, comparable to information on chemical constituents of the blood in health and disease, is available. For a general summary, including relatively old and also recent literature, the reader is referred to Wells[1] who presents a good critical review and bibilography.

Recently Gorecki[2] after examination of fairly large numbers of fluids for protein and various nonprotein constituents found that the proteins were high in exudates and low in transudates. Fatty acids were consistently higher in exudates and cholesterol moderately higher in exudates than in transudates. Sugar values were about the same as in blood in both types of serous fluids.

Bezancon et al[3] found slightly more chlorides in both transudates and exudates than in blood, whereas calcium values were practically the same as in blood. Fatty substances varied roughly with the protein content, which was increased in inflammatory exudates. The fibrin content was also highest in inflammations. Cholesterol was uniformly moderately higher in exudates than in transudates.

Bernhard[4] found on examining 14 fluids that the amount of nonprotein nitrogenous bodies and sugar content were comparable to the findings in blood. Nathan[5] states that the sugar content is slightly lower in inflammatory fluids than in transudates, a point denied by others, including Bezancon. Orsi[6] found that determinations of glucose were not of differential value in exudates and transudates.

A great deal has been written about the cytodiagnosis of fluids especially by French authors. The best résumé of the subject in English is found in Sahli's textbook[7] in which a critical discussion of Widal's original claims is presented. Briefly, Sahli's conclusions are that a sediment showing merely all lymphocytes suggests tuberculosis as the etiology of the effusion, but that fluids from other causes may show the same picture. He maintains that the predominance of one type of cell depends less on the primary etiology than on the stage or severity of the process. He advises hesitation in the diagnosis of tumor cells from smears alone, because of the danger of confusion with degenerated cells of other types, in which Bock[8] and Zemansky[9] agree, both

*From the Laboratories of the Buffalo City Hospital, Buffalo, N. Y.

Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minn., June 8, 9, and 11, 1928.

of whom recommend, as do many others, the sectioning of imbedded sediment and search for tumor particles. Miller[10] agrees fairly well with Sahli's general conclusions and gives his findings in a series of 75 fluids, studied as to cellular picture, specific gravity, gross appearance and protein content.

Unverricht[11] recommends the following methods of examining serous fluids from a standpoint of differential diagnosis: specific gravity, protein content, Rivalta test, presence of blood, cytodiagnosis, guinea pig injection. He, along with others, especially Sahli and Miller, call attention to the fact that some fluids show some of the properties of both transudate and exudate, a condition which may occur when there have been added some inflammatory changes to the serous surface in the case of a transudate, or when there has been absorption or marked pressure in the serous cavity. Unverricht calls attention to the fact that eosinophiles are sometimes seen after pneumothorax. Others have reported these cells in cases of postpneumonic pleurisy, tuberculosis (secondary), exudates of polyarthritis, pulmonary gangrene, malignant tumor, and following gunshot wounds of the chest (Simon[12]).

The subject of the serology of effusions has recently been discussed by Machailow[13] and Salomon and Valtis[14] who found that tuberculous fluids were satisfactory for the diagnosis of tuberculosis by the complement-fixation test.

Pousini[15] found that the Wassermann test for syphilis was identical in effusions and blood serum from the same patient.

Our own work consisted of the examination of 34 pleural and 29 abdominal fluids, most of which were delivered to the laboratory while still warm. These were studied by various physical, chemical, and cytologic methods with the aim in view of determining the features which would be of differential diagnosis from a clinical standpoint. Serous fluids only, free from pyogenic organisms, were used. The clinical diagnosis under which the fluids were grouped was corroborated by autopsy, operation, or guinea pig inoculation in about 60 per cent of the cases.

For cellular studies 15 to 30 c.c. of fluid were promptly centrifuged, and the sediment studied in wet films, unstained or tinted with neutral red or methylene blue, or dry smears stained by Wright or Giemsa's stain. A few sediments were imbedded in paraffin and sections examined for tumor particles.

Specific gravities of the fluids were taken with a standardized 15 c.c. urinometer at room temperature. Rivalta tests were conducted in the usual method, i.e., by pouring a few drops of the fluid into a cylinder containing 2 or 3 drops of glacial acetic acid in 100 c.c. distilled water. When positive a definite milky cloud was noted as the drops sank to the bottom. Controls with distilled water alone were used when the fluid was opalescent.

Guinea pigs were injected in all cases of suspected tuberculosis with the sediment of large amounts (6 to 8 oz.) of fluid, promptly centrifuged in 2 oz. bottles to obtain the sediment before clotting occurred. In some cases when clotting occurred the clots were squeezed free from the fluid and then ground in a mortar and injected with a large needle, along with the sediment. Anticoagulants such as citrate or oxalate were avoided, because of the toxic effect of the former on the animals and because the smears of the sediment were not

TABLE I

CHEST FLUIDS

TYPE OF FLUID	TOTAL FLUIDS		SP. GRAV.	RIVAL TA	FIBRIN	ALBUMIN %	GLOBULIN %	A/G RATIO	TOTAL N	PPN	UREA N	URIC ACID	CREATININE	CHLORIDES	SUGAR	CHOLESTEROL	LECITHIN	INORG P	CALCIUM
Primary T. B.	9	Max.	1025	4+	4+	3.24	2.89	1.95	904	34.7	16.1	4.2	1.5	650	116	212	367	4.5	10.3
		Min.	1020	3+	+	1.99	1.66	0.92	719	20.1	10.6	2.4	1.1	590	64	87	66	2.9	8.5
		Ave.	1021	3+	3+	2.67	2.12	1.31	796	25.9	13.6	3.2	1.3	609	92	134	127	3.5	9.2
Secondary T. B.	6	Max.	1024	4+	4+	3.03	3.73	1.39	823	31.8	16.1	3.4	2.1	655	122	210	92	5.0	13.9
		Min.	1019	3+	0	1.96	1.23	0.61	676	17.6	9.1	2.4	1.1	540	91	80	Tr.	3.4	8.4
		Ave.	1021	4+	3+	2.49	2.29	1.19	779	23.6	11.9	2.7	1.3	600	106	132	59	4.1	9.8
Non-T. B. Pleurisy	6	Max.	1025	4+	4+	3.12	3.11	1.49	988	29.7	18.1	4.0	1.7	660	99	286	125	4.1	9.8
		Min.	1018	+	+	2.50	1.68	0.94	728	16.9	9.8	2.0	1.1	570	70	141	Tr.	3.1	8.5
		Ave.	1022	2+	2+	2.82	2.32	1.26	837	22.7	12.6	2.9	1.4	584	83	194	67	3.6	9.2
Cardiac	6	Max.	1014	+	3+	1.22	1.23	3.51	339	42.5	39.1	5.0	2.8	695	328*	39	71.2	4.0	8.7
		Min.	1012	0	0	0.80	0.33	0.92	260	22.8	9.8	2.5	1.2	625	84	20	Tr.	3.3	7.9
		Ave.	1013	Tr.	+	0.97	0.79	1.50	287	34.8	28.7	4.0	2.0	668	153*	Insufficient no.			
Tumor	7	Max.	1025	4+	4+	3.30	1.67	2.84	773	36.8	22.4	6.8	1.9	690	118	137	82.7	6.1	10.7
		Min.	1018	+	Tr.	1.85	1.09	1.34	568	19.5	11.1	2.9	1.1	560	64	107	Tr.	3.4	8.7
		Ave.	1021	3+	2+	2.54	1.33	1.97	657	30.9	15.9	5.1	1.5	624	93	118	43.3	4.4	9.6

ABDOMINAL FLUIDS

Cirrhosis	10	Max.	1016	+	+	1.56	1.52	1.41	434	36.3	21.0	6.4	2.0	710	264	148	58.7	5.3	9.8
		Min.	1008	0	0	Tr.	Tr.	0.73	73	20.1	11.9	1.8	1.0	593	86	Tr.	Tr.	2.5	6.8
		Ave.	1012	Tr.	0	0.73	0.59	1.08	187	26.6	16.2	3.4	1.3	642	131*	46.8	19.3	4.1	8.5
Nephritis	3	Max.	1010	0	0	Tr.	Tr.	?	53	48.9	33.3	4.5	1.8	670	182	Tr.		5.4	7.2
		Min.	1009	0	0	Tr.	Tr.	?	45	28.4	23.1	3.0	1.4	530	125	Tr.		4.7	5.8
		Ave.	1010	0	0	Tr.	Tr.	?	49	39.9	27.1	3.9	1.6	620	147	Tr.		5.0	6.8
Cardiac	8	Max.	1018	Tr.	Tr.	1.64	1.69	1.62	554	42.8	25.9	5.3	1.6	660	182	127	140	4.4	9.9
		Min.	1008	0	0	0.32	0.21	0.91	129	21.5	13.3	3.5	1.1	600	121	5	22	3.1	7.5
		Ave.	1012	0	0	0.88	0.81	1.12	301	33.1	22.8	4.5	1.2	642	139	58	51	3.8	8.3
Tuberculosis	3	Max.	1023	4+	3+	2.19	3.55	0.93	890	29.4	25.1	4.2	1.6	605	96	95	92		
		Min.	1020	3+	2+	1.80	2.05	0.54	680	24.9	14.7	2.0	1.2	545	60	94	Tr.		
		Ave.	1022	3+	2+	1.96	2.65	0.78	775	27.3	21.6	3.1	1.3	577	78	94	34		
Carcinoma	5	Max.	1019	2+	2+	2.56	1.37	2.14	630	43.2	27.3	4.5	1.3	645	156	144	112	4.4	9.4
		Min.	1010	0	0	0.49	0.34	1.44	160	26.7	13.3	3.5	1.0	545	60	19	Tr.	3.1	6.6
		Ave.	1014	+	+	1.28	0.86	1.67	406	34.5	20.0	4.0	1.1	613	114	94	45	3.7	7.7

*High values in cases of diabetes mellitus with increased blood sugar.

entirely satisfactory when either was used. Prompt handling of the fluids obviated the use of these chemicals except in a few cases.

The chemical examinations were done by methods ordinarily used for blood and serum chemistry.

On analyzing the findings in the chest fluids (Table I) the specific gravity of the inflammatory exudates is seen to be much higher than that of the transudates in the cardiac congestive cases, varying from 1.019 to 1.025 in the tuberculosis cases (with an average of 1.0215), and 1.022 in the nontuberculosis inflammations compared with 1.012 to 1.014 (average 1.013) in the transudates. The tumor cases likewise were high, 1.018 to 1.025 (average 1.021).

The Rivalta test was consistently marked in the inflammations, most in tuberculosis or tumor, less in nontuberculous inflammations and very scant or negative in the transudates. Spontaneous clotting was most marked in the tuberculous cases, nearly as prominent in those with tumor, moderate in nontuberculous inflammations and only slight or not at all in the transudates.

Analysis of the chemical findings showed the protein content to be practically proportional to the specific gravity of the fluids. The exudates showed much higher albumin, globulin, and fibrin content than the transudates, averaging over three times the values in the latter class of fluids. Albumin-globulin ratios were not constant in any of the groups of fluids. Nonprotein nitrogen, urea, uric acid, creatinine, and chlorides showed values quite comparable to those of the blood. Many simultaneous determinations of these substances in the blood were nearly the same as those in the fluids, indicating free diffusion of these substances through the pleural wall into the chest cavity. The high figures for uric acid in the tumor cases possibly is due to the increased destruction of nucleoprotein.

The sugar content also paralleled the blood sugar quite constantly. In the cardiac group one fluid from a diabetic showed 328 mg. sugar, approximating the amount found in the blood.

Cholesterol values varied considerably in the different fluids with no data of differential value in any group of cases. The same applies to lecithin. Inorganic phosphorus and calcium values were practically the same in all types of fluids and were quite comparable to the amount of the same substances in blood.

Study of the cellular elements in the sediments as well as the fluids in the gross revealed blood in various amounts in all of the fluids. Most was seen in the tumor fluids, less in the tuberculosis cases, and least, amounting to microscopic blood only, in the nontuberculous inflammations. Occasional fluid showed a considerable amount of blood from trauma by the aspirating needle and no doubt traces seen in other fluids could be ascribed to this source.

Studies of the white cells in the tuberculosis fluids showed a marked preponderance of lymphocytes in all but 3 fluids, in which a considerable percentage of polymorphonuclears was seen. All three cases were very acute in onset and severity. The transudates showed characteristically serosal cells singly and in clumps, but in 2 cases with long-standing effusion a high percentage of lymphocytes occurred. Also, over 95 per cent lymphocytes were seen in one case of chronic postpneumonic, nontuberculous pleurisy and two of

lymphosarcoma of the chest (Fig. 1). In 4 other nontuberculous pleurisy fluids a high percentage of polymorphonuclears was present along with large numbers of large mononucleated cells, some of which were definitely macrophages showing phagocytosis.

All of the several fluids from cases with carcinomatous involvement of the pleura showed masses of large cells, irregular in size and shape, often vacuolated, and sometimes with mitotic figures (Fig. 2). Sections of imbedded sediment revealed small fragments of tumor tissue, especially gland acini in adenocarcinoma (Figs. 3 and 4).

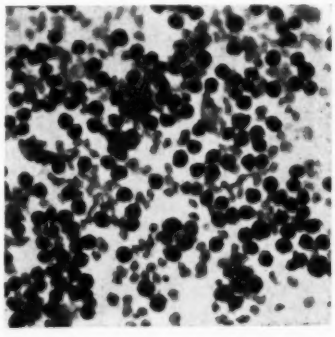


Fig. 1.

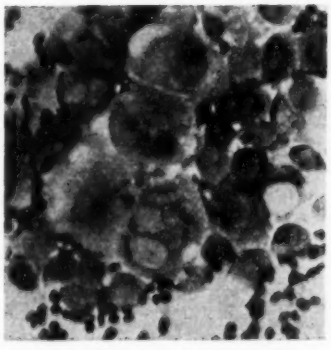


Fig. 2.

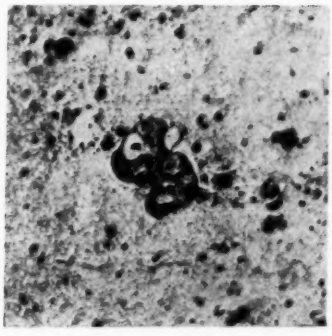


Fig. 3.

Fig. 1.—Lymphosarcoma. Cells indistinguishable from lymphocytes of blood.

Fig. 2.—Carcinoma metastases to chest. Note mass of irregular cells with prominent nucleoli.

Fig. 3.—Secondary carcinoma of pleura. Same case as Fig. 2. Section of imbedded sediment showing gland acinus.

Eosinophilia was seen in several fluids, a point to be discussed later (Table II).

Guinea pigs injected with sediments of several ounces of fluid from cases of tuberculous pleurisy were positive in 8 cases, 2 pigs died from too large a dose of sediment, and 3 were negative, a much higher percentage of positive results than if small amounts had been injected as sometimes recommended.

The results on abdominal fluids in Table I show much similarity to those of the chest fluids. The specific gravities were on the whole lower than in chest fluids with similar etiology, except in the cases of tuberculosis where the spe-

cific gravity averaged about 1.022. The transudates, whether due to cirrhosis, nephritis, heart disease, or mechanical obstruction in 3 cases of carcinoma showed low values, the majority about 1.012 or 1.013, with a few lower and some higher. The Rivalta test was negative or only faint in all the transudates and definitely positive in all the exudates, most marked in the tuberculous cases and moderate in two fluids from peritoneal carcinomatosis. The same applies to spontaneous clotting of the fluids.

Blood was found most abundant in 2 carcinomatosis cases and next in the tuberculosis fluids and was very scanty in all types of transudates, even in most of those of the cardiac decompensation group.

Fig. 4.—Piece of skin found in section of pleural fluid sediment, a "button" cut out by aspirating needle.

The chemical findings showed high protein values in the tuberculous and carcinomatosis cases similar to those in chest cases. The lowest figures were obtained in 3 fluids from 2 nephritic cases in which only traces of albumin and globulin were seen. Cirrhosis fluids were next to the lowest, and the cardiac fluids the highest of the transudates. The albumin-globulin ratio was inconstant, although in all the tuberculous cases the globulin was greater than the albumin. Fibrin was absent or scanty in all the transudates except one, and was most abundant in the tuberculous fluids.

Nonprotein nitrogen, urea, uric acid, creatinine, chlorides, and sugar approximated values found in blood, all cases having had some blood chemistry done, either some time before or shortly after paracentesis. However, the

uric acid values in many of the cases were somewhat increased over those of the blood. A few fluids with high sugar values were found in cases of diabetes with high blood sugar.

Cholesterol content varied within all groups of cases, although there was found only a trace in three fluids from two cases of nephritis. One of these cases, from whom two fluids were drawn, had a consistently high blood cholesterol, about 350 mg. per 100 c.c. of blood. Lecithin was not detected in the above three fluids. No constancy in regard to this substance was apparent in the other groups of cases.

The amount of inorganic phosphorus was of no differential value. Calcium determinations were fairly constant. Two nephritic fluids showed the lowest values, viz., 6.3 and 5.8 mg. respectively. One fluid from a case of cirrhosis with jaundice showed a low figure, 6.8 mg., as did a fluid from a case of carcinomatosis (6.6 mg.).

Cellular study of the transudates showed on the whole large numbers of classical serosal cells, singly and in plaques, in most cases comprising 50 per

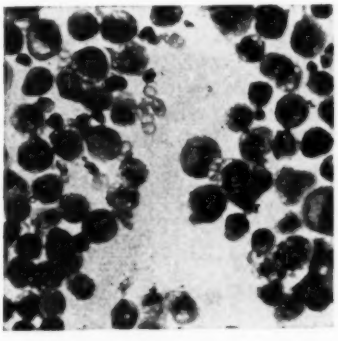


Fig. 5.

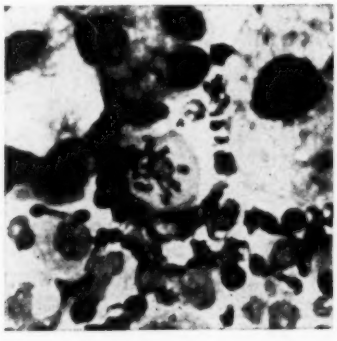


Fig. 6.

Fig. 5.—Carcinoma of peritoneum, secondary to stomach cancer. Note mitotic figures.
Fig. 6. Carcinoma of peritoneum, secondary to ovary cancer. Note large mass of irregular cells, one showing mitosis.

cent or more of the white cells seen. High lymphocytic values were seen in several cases of transudates of prolonged duration and in two tuberculous cases, not previously tapped. Three fluids from carcinoma patients with fluid apparently due to mechanical obstruction, showed pictures similar to those in other transudates. Cells similar to those described in tumorous fluids were found in the two fluids from cases with peritoneal metastases. Mitoses were seen in some of the cells in both fluids (Figs. 5 and 6).

Guinea pig inoculations were positive in all three fluids from the tuberculous cases.

Some attention was paid to several opalescent milky abdominal fluids, from nephritic and cardiac cases, in an attempt to determine the chemical nature of the substance causing the chyliform appearance. In only one, a cardiac case, was neutral fat demonstrated. Lecithin was highest in this fluid, but the values for this phospholipin in the other cases were no higher than in other fluids showing no opalescence.

TABLE II

CASE	DATE	PATIENT	NO. OF TAPS	LYMPHS	POLYS	LARGE MONOS	EOSINOPHILES	NOTES	CLINICAL DIAGNOSIS	TYPE OF FLUID
1	3-17-28	W.G.	1	25	20	50	5	L monos chiefly macrophages	Postpneumonic	Pleural exudate
	3-23-28	W.G.	2	20	5	15	60	L monos chiefly macrophages	Postpneumonic	Pleural exudate
2	4- 9-27	M.M.	1	30	30	40	0	L monos 90% serosal; 10% macrophages	Postpneumonic pleurisy	Exudate
	4-18-27	M.M.	2	30	25	30	15	L monos nearly all macrophages	Postpneumonic pleurisy	Exudate
3	4- 2-27	C.A.	1	10	75	10	5		Postpneumonic	Pleural exudate
4	7-11-27	J.R.	1	50	0	50	0	L monos all serosal cells	Cirrhosis of liver	Abdominal transudate
	8-20-27	J.R.	2	10	70	18	2	L monos partly macrophages	Cirrhosis of liver	Abdominal transudate
5	1-21-27	G.M.	1	50	0	50	0	L monos are serosal cells	Cirrhosis of liver	Transudate
	3- 5-27	G.M.	2	25	7	60	8	L monos are 50% large degenerated cells	Cirrhosis of liver	Transudate
	4-11-27	G.M.	3	30	5	60	5	L monos are 50% large degenerated cells	Cirrhosis of liver	Transudate
	7- 2-27	G.M.	5	10	25	50	15	Monos as before	Postmortem sample	Transudate
6	3-26-26	A.V.	2	23	50	25	2	L monos are 50% large degenerated forms	Tuberculous peritonitis	Abdominal exudate
7	12-22-26	F.B.	1	60	20	20	0	L monos chiefly large degenerated forms	Ca of lung	Pleural exudate
	1-20-27	F.B.	5	60	20	2	18	L monos chiefly large degenerated forms	Ca of lung	Pleural exudate
8	6-29-27	A.S.	4	1	93	1	5	T.B. Bacilli + in smear	TB pleurisy secondary	Pleural exudate
9	10-14-27	L.A.	5	2	20	3	75	Lung abscess	Pneumothorax (art.)	Pleural exudate
10	3-27-28	W.K.	1	45	5	50	0	L monos all serosal cells	Cardiac decompensation	Transudate
	3-29-28	W.K.	2	2	60	23	15	Some macrophages seen	Cardiac decompensation	Transudate
	4- 5-28	W.K.	3	5	10	25	60	Some macrophages seen	Cardiac decompensation	Transudate

In the other opalescent fluids the substance could not be shaken out with chloroform, ether, or alcohol. The same was true after warming with strong acid or with strong alkali. Compounds of a simple lipo-protein nature therefore seem excluded. Varying the P_H considerably on both sides of the neutral point was not effective in rendering the fluid clear. No definite conclusions were reached.

Wassermann tests were done on about a quarter of the chest and abdominal fluids and the reactions were similar to those with blood serum from the same patients. Of 10 chest fluids, sent to the New York State Laboratory at Albany for tuberculosis complement fixation, 6 were reported anticomplementary, 2 from tuberculous cases were positive and 2 from nontuberculous patients were negative. Further work along this line will be continued.

In observing repeated samples from the same patient in this series, and others not included here, a definite change in the cellular picture has been noted many times, characterized by the presence of polymorphonuclear neutrophiles and especially of eosinophiles (Fig. 7). The presence of this latter cell following repeated pneumothorax tappings has been repeat-

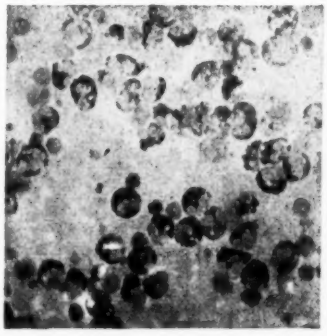


Fig. 7.—Post pneumonic pleurisy plus tapping. Note 60 per cent eosinophiles.

edly reported but their appearance following repeated taps in other conditions has not been described. In one case of cardiac decompensation 15 per cent eosinophiles and 60 per cent polymorphonuclears were found two days after a primary tap which showed the usual picture of a transudate with no eosinophiles and only a few polymorphonuclears. The highest percentage of eosinophiles we have ever observed is 75 per cent of the total cells, this occurring in a patient fourteen days after the first of 4 small injections of air given to compress a lung abscess. In none of these fluids have we been able to demonstrate organisms. Table II shows the counts in 10 fluids demonstrating the above phenomena.

SUMMARY

From the above results it is apparent that for differential diagnosis as to the type of fluid and the apparent etiology of an effusion no one method of examination can be used alone, but a combination of procedures must be used. Quantitation of the various chemical substances present, except the protein content, gave no real diagnostic information and even the latter was propor-

tional to the specific gravity of the fluids. Most of the chemical substances tested for, except proteins, were found in about the same amounts as in the circulatory blood. The Rivalta test was of particular value in determining transudates from exudates but in chronic transudates with some absorption of fluid or in cases with irritation from tapping, slight positive reactions were obtained. Spontaneous clotting was most marked in inflammations, but, like the Rivalta test, was seen if transudates remained long or were concentrated by absorption in the chest, or if irritation of the serous cavity by tapping, pneumothorax, etc., was an added insult to the lining membrane. Our experiences with cytodiagnosis agree fairly well with those of Sahli, namely, that the types of cells found depend on the stage of severity of the etiologic process, the duration of the effusion, secondary factors such as tapping, etc., as well as on the primary etiologic factor. Most cases of tuberculosis of the pleura, whether so-called primary or secondary, show 90 per cent or more of lymphocytes, but if very acute or if tubercle bacilli are abundant enough to be found by smears, polymorphonuclears may be found in various percentages. Some chronic nontuberculous pleurisies, chronic transudates, or even fluids from malignant conditions may show nearly exclusively lymphocytes in smears. In these fluids, however, the specific gravity, Rivalta test, protein content, and above all the clinical findings, will often help to classify the fluid.

The presence of masses of large cells, irregular in size and shape, often vacuolated, showing prominent nucleoli and sometimes mitotic figures is highly suggestive of malignancy, but definite diagnosis is better made by sections of the imbedded sediment, in which fragments of tumor tissue, especially gland acini in adenocarcinoma, can often be found. Confusion results in smear examinations when degenerated forms of large mononucleated cells, either serosal desquamations or cells of other types ordinarily designated as macrophages, are seen. Absolute classification of some of these cells is certainly impossible.

The presence of large amounts of blood, if trauma in aspiration can be ruled out, suggests tumor. Tuberculous fluids as a rule show enough blood to make the small button of sediment in a 15 c.c. centrifuge tube appear grossly bloody. Congestive transudates as seen in hydrothorax in heart disease show practically the same amount or less than the average tuberculous fluid.

Guinea pig inoculations for tuberculosis should be made with the sediment of many ounces of the fluid examined if positive results are to be obtained. The injection of 5 c.c. of fluid as recommended in many clinical and laboratory textbooks is usually unsatisfactory because of the fact that the bacilli are not abundant enough in such small quantities to infect a pig.

The presence of eosinophiles in the sediment of aspirated fluids in our experience has been found to be most commonly seen in cases in which repeated aspirations have been made, occasionally appearing promptly after a single tap. These cells have been seen many times in the fluid forming after injections of air in artificial pneumothorax treatment for tuberculosis or other lung conditions. Moderate numbers have also been found in the first fluid obtained in cases of postpneumonic pleurisies, especially in patients developing postoperative lung complications. The effect of tapping in producing this

cellular picture has not been stressed in literature, and possibly some of the cases of eosinophilia in the fluids recorded heretofore under the primary etiology of the effusion may be explained on the basis of repeated tapping. We have been unable to obtain postmortem material to observe the histologic changes in the serous surfaces in any of our cases, but probably there is an aseptic inflammation present. The concomitant presence of polymorphonuclears or their presence without eosinophiles would help to bear out this assumption. No organisms have been demonstrated in smears or cultures in any of the fluids referred to above. The effect of novocaine injected into the pleural surface or the pleural cavity should also be considered as a possible source of the cellular picture.

CONCLUSIONS

After final summary of the examination of the above fluids and others not included here we have concluded that the most practical points of differential value in endeavoring to classify an effusion as to type or primary etiology are as follows:

Gross description or appearance; presence and amount of blood; presence or absence of spontaneous clotting; specific gravity; Rivalta test; microscopic study of smears of sediment; study of sections of imbedded sediment in suspected malignancy; and guinea pig injection of sediment or clot from large amounts of fluid when tuberculosis is suspected. In very few fluids will only one of the above methods give conclusive evidence, but if the clinical features of the case with the history of the duration of the effusion, information as to previous aspirations, etc., are known, a fairly reliable diagnosis can be made if the various methods just mentioned are used. Chemical examination for substances other than protein is of no real diagnostic value. Possibly complement-fixation tests for tuberculosis may be worth consideration, a point now under investigation by us.

REFERENCES

1. Wells, H. G.: Chemical Pathology, ed. 4, Philadelphia, W. B. Saunders Co., p. 790, 1925.
2. Gorecki, Z.: Recherches Chimiques sur les liquides séreux, Rev. de Méd. **41**: 470-484, 1924.
3. Bezancon, F., Weil, M. P., and Guillaumin, C. O.: La Constitution Physico-Chimique des Liquides Pleuraux, Ann. de méd. **21**: 316-330, 1927.
4. Bernhard, A.: The Chemistry of Transudates and Exudates, With Reference to Their Nonprotein Nitrogenous Constituents, Interstate M. J. **25**: 188:191, 1918.
5. Nathan, H.: Ueber den Zuckergehalt Pathologischen Flüssigkeiten, Klin. Wchnschr., **6**: 1516-17, 1927.
6. Orsi, A.: Il Contenuto in Glucosio dei Liquidi Pathologici delle Grandi Sierose ed il suo Valare Diagnostico Pathologica. **18**: 482-484, 1926.
7. Sahli, Hermann: A Treatise on Diagnostic Methods of Examinations, Philadelphia, W. B. Saunders Co., p. 1229, 1914.

8. Bock, E.: Zur Mikroskopischen Diagnose von Geschwülsten der Pleura aus Punktionsflüssigkeit, Klin. Wchnschr. 4: 651-652, 1925.
9. Zemansky, A. P.: The Examination of Fluids for Tumor Cells, Am. J. M. Sc., 175: 489-504, 1928.
10. Miller, J. L.: Transudates and Exudates, With Report on 75 Fluids, Am. Med. 8: 835-843, 1904.
11. Unverricht: Die Klinisch Wichtigsten Methoden der Prüfung von Pleura- und Ascitesflüssigkeit, Klin. Wchnschr. 5: 2029-2030, 1926.
12. Simon, C. E.: A Manual of Clinical Diagnosis by Means of Laboratory Methods for Students, Hospital Physicians and Practitioners, ed. 9, Philadelphia, Lea & Febiger, p. 851, 1922.
13. Machailow, K.: Complement Fixation and Bacteriology of Exudates, Ztschr. f. Tuberk. 43: 191, 1925.
14. Salomon, M., and Valtis, J.: Recherche Comparative des Anticorps et de l'antigéne Tuberculeux dans le sang et les Liquides Pleuraux, Compt. rend. Soc. de Biol. 93: 1145-1146, 1925.
15. Pousini, G.: Sulla Reazione di Wassermann nei Liquidi da Versamento, Riforma Med. 42: 51-54, 1926.

DISCUSSION

Dr. Frederic E. Sondern.—I would like to ask Dr. Foord if he will tell us about the abdominal fluid from cases numbers 16 to 20; whether chyle was actually demonstrated, and if not whether he was able to determine the cause of the turbidity.

Dr. Kano Ikeda.—Some years ago in our laboratory we studied the chemistry of body fluids in comparison with blood chemistry. We found the same kind of results as Dr. Foord. The thing I would like to suggest here is this: We do a postmortem examination on a patient who has been in the hospital a very short time or whose diagnosis has been very obscure. On these cases it has been my custom to examine the pericardial fluid chemically. I have found at least one determination which is of value in arriving at a cause of death: namely, the determination of the fluid for urea nitrogen. Sometimes we have great difficulty in determining whether or not the patient had died of uremia. The urea nitrogen will show a marked increase in the pericardial fluid in uremic death. As far as the other constituents are concerned, I am not prepared to say. Sugar of course decreases very rapidly. If the sugar content remains, say 0.20 per cent, that gives us a clue that the patient may have had diabetes. I think it is a good practice for pathologists performing postmortems to do chemical analyses on the pericardial fluid. We find the same thing holds true with other body fluids.

Dr. Alvin G. Foord (closing).—In answer to Dr. Sondern I must say that probably I should have called the opalescent fluids "pseudo-chylous" rather than "chyliform," because of the difficulties in demonstrating fat content. Dr. Youngberg, as well as other chemists, has been able to learn very little about the cause of the milkiness of these fluids, and also in reading reviews on the subject, particularly as given in Weil's Chemical Pathology, there is found considerable disagreement on the subject.

In answer to the warning as to the diagnosis of tumor on single cells, I can say that I quite agree. As least I am not able to make such a diagnosis on tissues or serous fluids, although it appears to me that the latter would afford splendid opportunities for single cell study. The best way to handle sediments examined for tumor cells is by making sections of the imbedded sediment and searching the same for tumor particles, such as acini, etc. Once again I wish to reiterate that conclusions on fluids are best made after clinical study of the patient, and with a fair impression of the clinical problem involved, the laboratory evidence will be of more value.

Dr. A. H. Sanford.—We get a chocolate-colored fluid with endothelial cells in plaques in it and we guess that it is a case of malignancy. We have had men who have worked with Dr. MacCarty who know all about single cell diagnosis, examine such fluids. I never could get any of them to find any cells that they would call malignant. All of us have seen fluids that we think are malignant. We have not time to go into that but I think this is something important.

PRIMARY CARCINOMA OF THE FALLOPIAN TUBES*†

By Ernest Scott, B.Sc., M.D., and Marguerite G. Oliver, M.A.
Columbus, Ohio

THE first description of carcinoma of the fallopian tubes was given by Orthmann in 1886. Just forty years later, Wechsler[1] found 192 tubal carcinomas in the literature and added four additional cases. Since this article we have found seven other instances in the literature. Cameron[2] reported two, Ewing,[3] one, Beck,[4] one, Liang,[5] one, Heil,[6] one, Bower and Clark,[7] one, and we are adding two additional cases occurring in the surgical service of the White Cross Hospital and Ohio State University, bringing the total number of recorded cases to 201.

Therefore, it becomes evident that primary carcinoma of the fallopian tubes is a rather rare condition and that in comparison with the total pathologic lesions of this organ the percentage of malignancies is very small indeed. Of 19,000 gynecologic cases in Johns Hopkins Hospital (Vest[8]) only four instances of primary cancer were observed. Wechsler reported four cases out of 5,870 specimens at Lenox Hill Hospital, while the present two cases have occurred in a series of 5,000 surgical specimens. As the condition is often recognized only histologically, there are no doubt cases which have been overlooked where routine microscopic examinations were not made.

The literature of the subject has been so thoroughly covered by Wechsler in his recent article, that it is not the purpose of this paper to go into a complete comparative study, but to record only those cases that have occurred since his report and to add those occurring in our laboratories. Two of these cases have been bilateral, two have occurred in the right tube and three in the left.

Case 1.—The tissues under consideration were referred by Doctor Joseph Price of Mercy Hospital, with the diagnosis of an "old inflammatory process of the uterus, left ovary and tube." By his permission we are able to report this case.

Mrs. K., aged forty-seven years, was admitted to Mercy Hospital, Apirl 4, 1927, with a complaint of pain in the pelvis.

History.—The patient has had only the ordinary diseases of childhood but has always been in delicate health. There have been no operations and no history of venereal disease. Pneumonia at the age of sixteen years with complete recovery. At seventeen to eighteen years she had night sweats for two months. Menstruation started at the age of fourteen years of the twenty-three to twenty-four day type, often irregular but otherwise normal. There has been one normal delivery.

The present illness started with pain in the pelvic region in November, 1926. Treatment afforded no relief. About March 1, 1927, the pain became more severe and radiated into the groin and thigh, though limited to the left side. She complained of nocturia and

*Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minnesota, June 8, 9, and 11, 1928.

†From the Pathological Laboratories of Ohio State University and White Cross Hospital, Columbus, Ohio.

painful defecation, but her stools were free from blood. There was no vaginal discharge. Laboratory findings revealed a moderate anemia, otherwise nothing noteworthy. The preoperative diagnosis was multiple fibroids and an inflammatory mass on the left side of the pelvis. Panhysterectomy and appendectomy were done.

Macroscopic Description.—The specimen consisted of the uterus, right tube and ovary, which were sealed into a solid mass measuring 4.5 by 5 cm. No definite relationship of the tube and ovary could be made out; the tube was markedly distended, resembling an old pyosalpinx. On section the lumen of the tube was dilated, filled with a thick, yellow débris, and lined with short papillary outgrowths which showed numerous small areas of necrosis. The process did not suggest itself even at this time as being of a malignant nature.

The uterus was small, very firm in consistency. The endometrium was slightly thickened, measuring 4 mm. There were no tumor masses in the uterine wall or in the endometrium.

The opposite tube was normal in size, the fimbriated end was not sealed. The ovary was small with many scars over its surface.

Microscopic Findings.—Sections of the large mass show the tissue to be edematous and infiltrated with leucocytes, especially in small areas which simulated abscess formation. In addition to this inflammatory process the epithelium, which resembled the tubal mucosa, was decidedly hyperplastic, atypical in its method of growth and presented mitotic figures. There was a very definite infiltration of the muscular wall by masses of epithelial cells.

Sections from the uterus and cervix showed no invasion by the malignant growth.

Diagnosis.—

1. Primary papillary alveolar carcinoma of the left uterine tube.

CASE 2.—(A somewhat more typical history.) Mrs. L. S. S., aged sixty-one years, was admitted to White Cross Hospital on February 9, 1928, complaining of a bloody vaginal discharge and pain in the back.

The menses began at the age of thirteen years, were regular and normal. At the age of seventeen she became pregnant and aborted at three months, with normal recovery. There is no history of venereal disease. The menopause occurred at the age of forty-eight years when menstruation stopped completely. There was no further trouble until twelve years later, when she noticed a watery vaginal discharge which was sometimes bloody and at times profuse. She entered a hospital and a curettage of the uterus was performed. The curettments were found to be negative and she was assured at this time that she would entirely recover. However, since that time, she has complained of constant discharge which upon numerous occasions has become bloody. She has also complained of continual pain in her back during this period. There has been no loss of weight and otherwise the patient has been in apparently normal health.

The first of February, 1928, the discharge had become more profuse. On pelvic examination a slight tenderness could be elicited over the region of the left tube, though no definite diagnosis could be made.

On February 9, she entered the hospital at which time the Wassermann and Kahn tests were negative. Her pulse was 80, temperature 98, blood count, blood chemistry, and urinalysis were not unusual, other than a slight leucopenia. Her appetite was good and she complained of no constipation. She has urinated twice a night during the last few months.

An exploratory operation was advised with a preoperative diagnosis of left tubo-ovarian infection or uterine neoplasm.

Operative Notes.—The uterus and cervix were small and atrophic. There was a large amount of periuterine adhesions. The right tube and ovary were apparently normal. The left tube was markedly enlarged, soft and cyst-like, and the fimbriated end sealed.

A left salpingo-oophorectomy and a right salpingectomy were performed.

Macroscopic Findings.—The specimen consisted of the right and left fallopian tubes and a portion of the left ovary. The ovary measured 2 cm. by 1½ cm. by 1 cm., and was somewhat atrophic. On section there was revealed three or four small follicular cysts. There

was no evidence of tumor nodules. Upon opening the left tube a large papillomatous outgrowth was revealed covering the entire inner surface of the tubal wall and everting its edges. The tube measured 8 cm. by 6 cm. by 6 cm. The tumor mass consisted of multiple long finger-like projections of the tubal mucosa, greyish white in color, arborescent and very friable. There was a small amount of serous exudate within the lumen and occasional dark areas of hemorrhage. Necrosis was not marked. The tubal musculature was thickened and firm. The peritoneal coat presented a few fibrous adhesions.

The opposite tube measures 5½ cm. in length by 1½ cm. in diameter and was kinked and tortuous. On section the wall was generally thickened by fibrous tissue and the mucous membrane was absent.

Microscopic Findings.—Section of the left fallopian tube, throughout its enlarged portion, revealed numerous papillary outgrowths of the tubal folds, consisting of a central stroma covered with cubidal and polyhedral epithelium which occurred in a single or multiple layers and in some areas in the form of solid cellular strands. Mitosis was abundant. Imbedded deeply in the musculature of the tube were masses of epithelial cells, some in alveolar arrangement, some in solid masses in all regards similar to the picture seen in gastric carcinoma. The serous coat in no instance was invaded. There was a marked round cell infiltration of the muscle wall.

The right tube showed marked fibrosis of the muscularis with obliteration of the mucous folds.

The ovary revealed no invasion of malignant cells.

Diagnosis.—

1. Papillary carcinoma of the left fallopian tube.
2. Right chronic salpingitis.

Note.—This patient returned to the hospital, eleven months following removal of the carcinomatous tube, presenting three nodular masses in the abdominal scar, measuring 2 cm. in diameter. The lower abdomen was painful and tender. Upon opening the abdomen the omentum was found to consist of a mass of carcinomatous nodules closely attached to the transverse colon. Both the visceral and parietal layers of the peritoneum were extensively studded with similar nodules. The uterus itself was small and free from invasions. The metastatic nodules were limited to the lower portion of the abdomen and mesenteric nodes.

Pathology.—Macroscopic examination revealed numerous soft, whitish tumor masses which were papillomatous and friable. The microscope presented the picture of adenocarcinoma, resembling, in close detail, that described as occurring in the fallopian tube eleven months previously. The case was inoperable and the patient left the hospital with a hopeless prognosis.

The most frequent and pronounced symptoms of carcinoma of the fallopian tube are, a profuse serous, often bloody or serosanguinous, vaginal discharge, pain, irregularity of the menses in younger women and negative curettements. Tumor masses may or may not be palpated. Constipation, nocturia, anemia and weakness are frequent associations in the latter part of the disease.

Both of the cases here reported fell in the postclimacteric period. One case presented a persistent serous and spasmodically bloody discharge of a year's duration, pain in the back and tenderness in the left lower quadrant of the abdomen, while the other was entirely free from any discharge but in neither of these cases, was the malignant nature of the disease suspected before operation.

SUMMARY

1. In this paper we have reported seven additional cases of primary carcinoma of the fallopian tube in the literature and two occurring in our own laboratory, bringing the total number reported to 201.

2. The most common clinical symptoms of the disease are those of vaginal discharge, often bloody, pain in the pelvis, tumor mass, and, late in the disease, constipation and nocturia.

3. The gross appearance of tubal carcinoma is often deceiving and its presence may be entirely overlooked unless careful routine microscopic examinations are made.

4. The microscopic picture is usually that of papillary-alveolar carcinoma.

REFERENCES

1. Wechsler, H. F.: Primary Carcinoma of the Fallopian Tubes, Arch. Path. & Lab. Med. 2: 161-205, 1926.
2. Cameron, S. J.: Malignant Disease of the Ovaries and Fallopian Tubes, Brit. M. J. 2: 285-288, 1925.
3. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1127, 1928.
4. Beck, Walter: Primares Tubenkarzinom, Zentralbl. f. Gynäk. 50: 1503-1505, 1926.
5. Liang, Z.: Was lehrt das primäre Tubencarcinomen pathologischer Hinsicht? Virchow's Arch. f. path. Anat. 259: 576-607, 1926.
6. Heil, Karl: Primares Tubenkarzinom, Zentralbl. f. Gynäk. 50: 2952-2958, 1926.
7. Bower, J. O., and Clark, J. H.: Primary Bilateral Carcinoma of the Fallopian Tubes, Arch. Surg. 11: 586-597, 1925.
8. Vest, Cecil W.: A Clinical Study of Primary Carcinoma of the Fallopian Tubes, Bull. Johns Hopkins Hosp. 25: 305-317, 1914.

DISCUSSION

Dr. A. S. Giordano.—I had one case like that. I had never seen one before and was very doubtful about saying what it was.

Dr. Clarence I. Owen.—I would like to ask about recurrence and metastasis.

Dr. Ernest Scott (closing).—One of the cases died a few days after operation and the other was operated upon only sixty days ago.

THE INTERPRETATION OF BORDERLINE ALLERGIC REACTIONS*

By Warren T. Vaughan, M.D., Richmond, Va.

THE interpretation of allergic skin reactions has passed through a variety of evolutionary phases. Even now the procedure has recognized shortcomings.

THE DELAYED REACTION

At the beginning the immediate or half-hour reaction was alone recorded and no reaction was considered positive by the scratch method unless accompanied by an urticarial wheal preferably with pseudopod formation and surrounding erythema. In 1922 and 1923 Peshkin and Rost,[1] Sidlick and Knowles,[2] Shannon,[3] Vaughan,[4] and others called attention to delayed types of the reaction usually showing up after an interval of about twenty-four hours.

Vaughan suggested that the mild immediate reaction and the delayed positive reaction constituted the logical type of response in those cases in which the protein poisoning was chronic as with foods eaten daily, and that these reactions were the clinical counterpart of the well-known experimental Arthus phenomenon. The latter represents chronic protein poisoning following daily local injections of horse serum, manifesting itself as a soft infiltration which later becomes hard, edematous and even necrotic.

Most allergists I believe now make the twenty-four-hour reading along with the half-hour study. A good positive delayed reaction is never as pronounced as the strongly positive immediate reaction, never progressing to the formation of an urticarial wheal but it may be much more strongly positive than was the immediate reaction to the same protein. Indeed a good positive delayed reaction may follow an entirely negative immediate reaction. The delayed reaction consists of slight induration along the line of scratch with surrounding erythema usually rather sharply demarcated, of from one to five centimeters diameter. It looks rather like a beginning infection but fails to progress as such.

Not infrequently persons known through experience to be sensitive to certain proteins display persistently negative skin reactions to these same proteins. Many such cases reported negative to the immediate reaction would have shown positive delayed reactions. The man who makes only half-hourly readings will miss the not infrequent delayed positive reaction, a reaction which I consider equally as specific as the immediate one, and will erroneously conclude that the reaction to the protein was entirely negative.

The selection of twenty-four hours as the time interval for the reading of the delayed reaction is purely arbitrary. The reaction is usually pretty well faded by the end of forty-eight hours but in rare instances it will persist two or even three days. It is not necessarily at its maximum after twenty-four

*Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minnesota, June 8, 9, and 11, 1928.

hours. It may already be fading. I have sometimes obtained negative or nearly negative half-hour and twenty-four-hour reactions with a clearly positive response at the end of four to six hours. I therefore make it a routine procedure to take three readings, the first at the end of twenty to thirty minutes, the second after from four to six hours and the third on the following day. With this routine one is very likely to catch the delayed positive reaction at some stage in its evolution. Even then the maximum reaction may have been missed. I have seen cases in which the maximum response occurred at the end of two hours but the four- to six-hour reading was still sufficiently strong so that the value of the reading was not lost.

BORDERLINE REACTIONS

The man who insists upon a clear-cut positive reaction and fails to consider the borderline response will often miss the specific allergenic protein. The response is not necessarily maximal. The scratch method is rather a crude procedure but when carefully carried out is surprisingly accurate as checked by repetitions of the test. But we cannot be certain that enough protein has passed into solution or enough has actually come into contact with the tissue cells to provoke a maximum response. Perhaps there was just enough to give only a mild reaction. I have often seen the drop of dissolved protein spread over an area of half an inch or more surrounding the scratch, the manipulator evidently failing to realize that only along the line of the abrasion is one capable of eliciting any response.

We know that immediately following an acute exacerbation negative responses are often obtained to proteins which at other times give positive reactions in the same individual. I do not imagine that a patient in such a negative phase changes suddenly from being a negative reactor to a strongly positive one. There are undoubtedly gradations during which the reaction may be borderline.

GRADES OF REACTION

The immediate reaction is the one of greatest value but the early and late delayed reactions give sufficient information so that we cannot afford to overlook them. An immediate reaction with a definite urticarial wheal and usually some surrounding erythema I class as one-plus. More strongly positive reactions are two-plus, three-plus, etc. The borderline immediate reaction with no wheal but with more erythema than those of its neighbors is plus minus. I even designate a third group, those in which the reaction can scarcely even be called questionable as plus minus minus. These are just sufficiently different from their neighbors to be termed barely suggestive. The one-plus delayed reaction shows an erythema of say one or one and one-half centimeters in diameter. Above this we observe two-plus and three-plus reactions and below it as on the immediate, questionable or plus minus and barely suggestive or plus minus minus reactions.

An isolated plus minus minus reaction receives no further consideration. But if on all three readings which have been made without reference to each other a particular protein shows up as plus minus minus each of the three times, the protein may be one of etiologic significance and is so considered.

In the same way a plus minus reaction on two or three of the three readings is given consideration and an experimental trial.

In this way I believe that I have increased my efficiency in detecting etiologic proteins. Many of the proteins incriminated in this manner are later found not to be factors in the individual case but others which have given only borderline reactions have sometimes been shown unequivocally responsible for symptoms.

ANALYSIS OF READINGS IN A SERIES OF BENEFITED CASES

A small series of 39 eczema cases, all relieved by protein avoidance only, was analyzed as to the character of the reactions. Clearly positive immediate reactions occurred in 61.5 per cent. Thirty-five per cent presented clearly positive delayed reactions. Often the immediate and delayed reactions occurred in the same individual, but in 17.9 per cent the delayed reaction was relatively more intense than the immediate. In 17.9 per cent the diagnosis was based entirely upon delayed reactions, the immediate being no stronger than plus minus. Of these latter cases four showed the strongest reaction at the end of from four to six hours and three at the end of twenty-four hours.

RESULTS OF TREATMENT IN BORDERLINE AND DELAYED REACTORS

In 1926[5] I reviewed a series of 135 allergics comprising groups of respiratory and cutaneous allergy (Table I). One hundred and thirty-two had had the complete routine skin test with three readings. Of these, 46 per cent showed a clearly positive immediate reaction, 7 per cent a clearly positive delayed reaction, and 47 per cent gave at best only borderline reactions on all three readings (Table II).

TABLE I

ALLERGIC DISEASES STUDIED

DISEASE	NO. CASES	NO. BENEFITED	PER CENT BENEFITED
Asthma	48	32	68
Eczema	28	15	54
Pruritus ani	22	9	41
Vasomotor rhinitis	15	11	73
Urticaria	22	16	73
Total	135		61.5

TABLE II

DISTRIBUTION OF CASES ACCORDING TO CHARACTER OF ORIGINAL SKIN TEST

	CASES	PER CENT
Good Prompt Reaction	61	46
Good Delayed Reaction	9	7
Borderline Reaction	62	47
Total	132	100

The question naturally arises as to which of these three groups experienced greatest benefit, those with good prompt reactions, those with good delayed reactions, or those with poor reactions. Table III answers this question. Table IV is but a condensation of Table III in which the results are grouped and may be more readily read. In the third column in Table III those receiv-

ing but 50 per cent improvement are included since I feel that with no other treatment prescribed these individuals may attribute such improvement as they have had directly to protein avoidance even though the results in the 50 per cent group have not been sufficiently good so that the patient was satisfied.

TABLE III

PROGNOSTIC VALUE OF SKIN TESTS
COMPARISON OF ORIGINAL SKIN TESTS WITH ULTIMATE RESULTS

EXTENT OF IMPROVEMENT	NONE	SLIGHT	DISTINCT	VERY GOOD	COMPLETE RELIEF
	(0%)	(25%)	(50%)	(75%)	(100%)
Good Prompt Reaction (61 cases)	18	15	13	36	18
Good Delayed Reaction (9 cases)	22	11	11		56
Borderline Reaction (62 cases)	37	13	16	19	15
Total, 132 cases	27	14	14	26	19

TABLE IV

PROGNOSTIC VALUE OF SKIN TESTS
(Continued)

EXTENT OF IMPROVEMENT	UNSATISFACTORY	VERY SATISFACTORY	DIRECT BENEFIT
	(0-25%)	(75-100%)	(50,-75-100%)
Good Prompt Reaction	33	54	67
Good Delayed Reaction	33	56	67
Borderline Reaction	50	34	50
Total	41	45	59

TABLE V

DISTRIBUTION OF REACTIONS ACCORDING TO END-RESULTS

NO.		PROMPT REACTION PER CENT	DELAYED REACTION PER CENT	GOOD REACTION PER CENT	POOR REACTION PER CENT
36	Unimproved	31	5	36	64
54	Little benefit	37	5.5	47.5	58.5
59	Very satisfactory	56	8	64	36
25	Entire relief	44	20	64	36

Referring for the present purpose only to the first two columns we see that with good prompt reactions and good delayed reactions the percentage of satisfactory results is decidedly higher than that of unsatisfactory results. Indeed, for every patient not benefited there were two who experienced appreciable measures of relief directly attributable to the protein avoidance.

But the significant fact brought out in this table is in the last group, those showing only borderline reactions. Thirty-four per cent of these obtained very satisfactory results from protein avoidance only. For every borderline reactor unimproved there was another which showed evidence of direct benefit. This would appear to substantiate my claim that in many clinics not enough attention is being paid to the borderline reactions.

ANALYSIS OF UNIMPROVED CASES

Table V brings out a new observation. Of those cases who received no benefit whatsoever 36 per cent had given a good clear-cut positive sensitization reaction. Why did these good reactors show no improvement following specific protein avoidance?

In 1927[6] I made a study of 71 consecutive cases of eczema. Thirty-nine or 55 per cent were relieved of the eczema by protein avoidance only. Forty-five per cent were not sufficiently relieved to consider the procedure satisfactory. Further study of these two groups, the *good result* and the *poor result* series, brings out some interesting facts.

Clearly positive prompt reactions were obtained in 61.5 per cent of the 39 good result cases. But they were obtained in almost as high proportion in the poor result cases, namely, 50 per cent.

Complete past and family histories were obtained in 39 of the entire series, 19 being good result cases and 20 poor results. Eight of the former gave a positive past history for one or more other allergic diseases. But eight of the latter gave equally definite positive histories.

Eleven of eighteen good result cases gave a positive *family* allergic history. Against this fourteen of the nineteen poor result cases in which family history was obtained gave a positive family history for allergy.

Seven of eighteen good result cases gave a family history of eczema and six of nineteen among the poor results did likewise.

A clear-cut delayed positive reaction was found in seventeen of thirty-nine good result cases and seventeen of thirty-two with poor results.

Although one series obtained satisfactory relief from protein avoidance and the other little or none, the evidence just recorded suggests strongly that allergy is a factor even in those cases which were not relieved.

TECHNIC

A word with regard to the method of making the test. The arm and forearm are still used for scratch or intradermal testing by the majority of allergists. I have discarded this site some time ago in favor of the back. The individual nerve terminations are more widely separated in the skin of the back than elsewhere and the back is therefore less sensitive to pain. The patient does not see the scratching or needling and the psychic pain factor is thus to a great extent eliminated. This is especially helpful in children. I have compared the strength of the reaction in a small series of known reactors, on the back, the arm, the forearm, and the thigh, and have found no great variation in the intensity of reaction in these areas. Six cases were studied in all. The reaction on the back was as large as or larger than elsewhere in every case except one. Even in this latter it was clearly positive. The comparison was rather rough since the scratch method was used but this method was chosen since it is precisely that which is used in the actual work of testing.

Alexander[7] has studied the reactivity of the skin to test proteins at various sites of the body with carefully controlled experiments using intradermal inoculations of very carefully measured amounts and always in duplicate. In such places as the forearm, the back, abdomen, the leg, and the thigh he finds varying cutaneous response or sensitiveness depending upon the location even when the tests are practically simultaneously applied. The skin of the back and of the abdomen usually gave a slightly stronger reaction than the other areas mentioned although occasionally the reverse was the case.

In experimental work with histamine, using dogs, he found some variation in sensitivity of the skin in different areas

Any number of tests may be applied on the back at one sitting, one hundred being easily given with no discomfort to the patient and no crowding of the scratches. The esthetic advantage of scratches in a location where they will not show are appreciated especially by the feminine allergics.

There is no more justification for applying the temporarily disfiguring skin tests to the arms than there is for producing a great smallpox scar in a locality that is often exposed. Doctors are great creatures of habit.

Fig. 1 illustrates the method of the dorsal application and brings out how much easier it is to apply the scratches or the intradermal test to one continuous surface rather than to upper and lower circumferences of two arms. In the case of men the factor of extensive distribution of hair does not so often require circumvention.

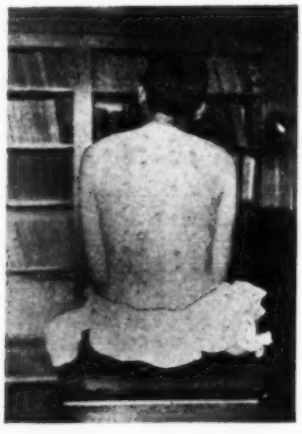


Fig. 1.—Illustrating method of applying sensitization tests to the back at one sitting.

Most writers recommend a one-eighth inch scratch for the application of the protein. Such a short scratch may be esthetic but it lessens the probability of sufficient protein coming in contact with the tissue cells to produce reaction. My scratches are about three-eighths of an inch long. I find that with these larger scratches the delayed reaction particularly is more easily read. I have yet to see a case of infection from the application of the protein test either by scratch or intradermally.

In the event of borderline reactions, we are left two alternatives, therapeutic trial by elimination and subsequent exposure, or the intradermal test. The intradermal reaction is often positive when the scratch has been negative, particularly in adults. This latter alternative should be the method of choice especially in those cases where specific desensitization is contemplated. Where there is reason to suspect a given protein, even the intradermal reaction should not be considered absolutely negative until after the application of a highly concentrated test extract.

Occasionally the reactions to scratch and to intradermal inoculations are so mild and yet the history is so definite that subcutaneous and ophthalmic

reactions become necessary. Many writers have mentioned the subcutaneous method, the more recent writers being Kahn and Grothaus[8] and Balyeat.[9] The former authors find that in Texas where pollen prevalence is extremely high through most of the year, persons with such mild sensitiveness will not react by scratch or intradermally, nevertheless they are specifically sensitive to pollens, a fact which can be demonstrated by the subcutaneous reaction.

CONCLUSIONS

There are many factors to be considered in the performance and interpretation of sensitization tests but I would emphasize that if two delayed readings are made in every case, pollens excepted, if more attention be paid to the borderline reaction especially when it occurs on two or more of the three readings; if the scratch be made the proper length; and if questionable scratch reactions are checked by intradermal tests, there will be fewer false negative reports, there will be fewer occasions for repeating the entire series, and therapeutic efficiency will be increased.

REFERENCES

1. Peshkin, and Rost: Am. J. Dis. Child. 23: 51, 1922.
2. Sidlick, and Knowles: Am. J. Dis. Child. 23: 316, 1922.
3. Shannon: Ibid, p. 392.
4. Vaughan, W. T.: South. M. J. 17: 749, October, 1924.
5. Vaughan, W. T.: South. Med. & Surg., July, 1926.
6. Vaughan, W. T.: J. Lab. & Clin. Med. 13: 24, Oct., 1927.
7. Alexander, Harry L.: Communication Before the Association for the Study of Asthma and Allied Conditions, Washington, D. C., May 28, 1928.
8. Kahn, I. S., and Grothaus, Emma M.: M. J. & Rec., March 3, 1926.
9. Balyeat, Ray M.: J. Oklahoma M. A., August, 1926.

707 Medical Arts Building.

DISCUSSION

Dr. J. H. Black.—Dr. Vaughan's paper, I think, is of value because it brings up two points which are very important. First, it proves very definitely that a lot of literature that is being sent out is quite fallacious. Literature is sent out giving the idea that the tests are perfectly easy to interpret. I think this type of paper illustrates very definitely that this is not true. Second, men doing this type of work are doing it in as many different ways as we are doing Wassermanns. Some are doing intradermal and some cutaneous tests. There is no uniformity as to the method in use. There is also a great deal of difference in the method of reporting results.

The interesting thing in this work is the possible relationship of his delayed reaction to the absence of "allergic antibody" in the blood. We have found that certain individuals who were entirely negative to intradermal injection, showed no antibody in the blood. We have believed that the negative reaction depended upon the absence of antibody. I should like to ask if Dr. Vaughan has made any effort to determine whether this is the case with those showing delayed reaction and also whether he finds delayed reactions in patients giving a negative intradermal reaction.

Dr. Wm. G. Exton.—I would like to ask Dr. Vaughan as an allergist for some explanation of the wheals we occasionally see immediately after inserting the needle into the veins of the arm in order to get blood specimens. They have interested me for a number of years. Do cleaning fluids have anything to do with it or is it just a reaction from the needle prick? Or what other explanation would an allergist give?

Dr. A. H. Sanford.—I should like to ask Dr. Vaughan to discuss something that may be what Dr. Exton refers to; that is what his procedure is in the hypersensitive skin especially with intradermal tests where you get a reaction to everything including the control.

Dr. Warren T. Vaughan (closing).—With regard to Dr. Exton's question, at the bend of the elbow you get more reaction than either above or below, and we avoid the bend of the elbow. I feel that most of this is purely traumatic. The tissue in the bend of the elbow is much looser than the skin above and below, and there is more room for swelling. If you get too near the elbow you may get false positives.

I have not, Dr. Black, made any special studies along that line. There was an interesting paper presented at the Washington meeting of the Asthma Society which might bear short repetition. It came from Philadelphia, bearing on the use of immune serum. A positive skin reaction to horse serum does not necessarily mean that the patient will get asthma after serum injection because the reactivity might be in the skin alone. When a positive skin reaction was obtained on the patient, the technic was to take the blood from the patient, inoculate it intradermally into another individual, and make a skin test at the site of intradermal inoculation. If you again get a positive reaction the "reagins" are also in the patient's blood. Then, of course, the patient may get asthma as well as the hives, following serum treatment.

We do see delayed positive intradermal reactions, which are sometimes more clearly positive than the immediate reaction to the same protein.

The nonspecific reaction is a problem. I do not know of any solution as yet. You will notice that in the picture of the back there were a lot of reactions. Sometimes where you have general cutaneous irritability there is enough variation in the degree of reaction to give you a lead and you can try out therapy on the basis of relative size of reactions. Sometimes the delayed reaction will help a great deal in these general reactors. There is less tendency for nonspecific generalized delayed skin reactions. Occasionally a series of peptone injections will lessen the nonspecific tendency so that later skin testing will bring out only the truly positive substances.

A METHOD FOR MEASURING THE BACTERICIDAL ACTION OF WHOLE BLOOD AGAINST GRAM POSITIVE COCCI*†

By William Thalhimer, M.D., and Charlotte Colwell, A.B.,
Milwaukee, Wis.

THIS report deals with the development of a method for accurately determining the ability of whole blood to kill gram positive cocci in vitro, particularly Staphylococcus aureus. When this investigation was started, a simple and accurate method of this sort was not available. An accurate bactericidal method is needed, since many investigators have found that the presence in blood of antibodies, such as agglutinins, precipitins, complement-fixing bodies, and others, does not indicate the degree of bactericidal power of the blood. It is evident that many clinical problems and problems in immunity which are in need of investigation can be studied with the aid of a proper method.

The problem of determining the bactericidal power of blood, or even of serum, against gram negative organisms, such as B. typhosus, is a comparatively simple one, and methods for this have been available for a long time. The method of Denison[1] is excellent, and soon after it was described one of us (W. T.) used it in an attempt to determine the bactericidal power of blood and serum against gram positive cocci. In these unpublished experiments, whereas the results of Denison were corroborated, no killing effect was found on gram positive cocci. Whole blood, kept from clotting by potassium oxalate and small amounts of hirudin, and serum, with and without the addition of guinea pig serum, were studied. In the light of the experience gained in the present study, the most probable explanation of the former negative results is that the blood was diluted and was not allowed a sufficient period of time to act on the bacteria before the mixtures were plated.

The failure of Denison's method with gram positive cocci should not be considered in any way as a reflection on this method when used for gram negative bacilli. Denison's method does not seem to have gained the prominence which it deserves.

About two years ago, at the request of Dr. C. H. Davis, a study of the bactericidal power of blood was made, using the method described by Heist and his coworkers.[2] In this method five capillary tubes are used, into which are introduced the same amount of different suspensions of pneumococci. Each of these suspensions is a 1.5 dilution of the previous one. The fluid is then drawn or blown out of each tube, leaving some pneumococci deposited on the inside of the wall. Immediately after this each tube is filled with whole, unclotted blood to the same mark used to measure the pneumococcus suspension. The end of the tube is sealed with paraffin, and the tubes are incubated for twenty-

*From the Laboratories of Columbia Hospital, Milwaukee, Wisconsin.
†Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minnesota, June 8, 9, and 11, 1928.

four hours. At the end of this time the blood-bacterial mixtures are blown out on a series of slides, dried, and stained for bacteria, so as to determine whether or not the bacteria have increased or decreased in number, and the degree to which this has occurred. We found, as did Matsunami and Kolmer,[3] that one can detect gross differences in the bactericidal activity of whole blood by this method. We found, as did these workers, that we could not detect slight differences in bactericidal power, nor did the results in duplicate tests check sufficiently close to one another to establish confidence in the method.

We then abandoned this method and did not resume our studies until December, 1926, at the request of J. L. Yates. At this time the chief object was to devise a bactericidal test that could be used for determining the best time for using donors in performing immuno-transfusions.* At this time, although we applied the principles of Heist's capillary tube method, we worked with amounts of whole blood and bacterial suspension large enough to be measured accurately with pipettes.

The first problem was to secure whole sterile blood that would not clot. This was easily solved by using small amounts of the anticoagulant heparin, as this substance can be sterilized readily by boiling or autoclaving. Other reasons for the choice of heparin were that it was inconceivable that such small amounts as were necessary to keep blood from clotting could alter in any way the bactericidal power of the blood, and because other substances, such as potassium citrate or oxalate, are believed by Colebrook and Storer[4] to decrease the bactericidal power of blood.

Following the scheme of Heist, our first tests were made using different samples of the same blood seeded with different numbers of Staphylococcus aureus. This method Sir A. E. Wright[5] has called "the implanting and explanting procedure," in contradistinction to the implanting and inculturing procedure.

A measured amount, 0.5 c.c., of each blood-bacterial mixture was inoculated immediately into a tube of melted agar, plated, incubated, and the colonies counted. This gave the essential control. The blood-bacterial mixtures were put in the thermostat and 0.5 c.c. was removed at two-, four-, six-, and eight-hour intervals, and plated in the same manner as the control. The flask containing the blood-bacterial mixture was thoroughly shaken immediately before each sample was removed. Our early tests showed a marked diminution of living organisms at the end of four hours' incubation. For a while we arbitrarily used this interval in the tests, but continued to use several samples of blood, each with a different number of bacteria. (See Table I.)

The first test yielded a very unexpected finding. Although there were 5 times as many bacteria in the third set-up as in the fourth, and 20 times as many as in the fifth, the percentage of organisms killed in all 3 set-ups was approximately the same. For example, in the first set-up with the greatest number of bacteria, the control plate contained approximately 10,000 colonies, whereas in the plate poured at the end of two hours the number of colonies was reduced to 2500, or to 25 per cent of the control. However, in the third

*The progress of this investigation of immuno-transfusions is reported in the paper, "Estimating the Increment in Bactericidal Power of Individuals' Blood Produced by Intravenous Injection of Typhoid Vaccine," by Charlotte Colwell and J. L. Yates.

set-up containing the smallest number of bacteria, there were 432 colonies in the control and 126 colonies in the two-hour plate, or a reduction to 29 per cent of the control. Therefore, in the first instance 7500 colonies disappeared, and although there were only 432 organisms in the second test, 29 per cent of these remained alive. These findings have been verified many times, and are entirely inexplicable at present. The experiments have been verified by using in different set-ups with the same blood organisms which varied from about 100 in each 0.5 c.c. of blood to about 10,000, which is about the limit of the number that can be accurately counted on a plate. In each instance the percentage of organisms which disappeared, based on the number found in the control plate, was always approximately the same. The killing power of the blood in these in vitro tests does not seem to follow a quantitative law, but a law of percentages. It is interesting that Sir A. E. Wright, with his slide cell method (of implanting and in-culturing) found the same percentage relationships and absence of quantitative relationships. This will be discussed again later in this communication. We did not observe, however, the phenomenon which Wright called "epiphylaxis," that is, a larger percentage of organisms being killed when a larger actual number are present in the blood-bacterial mixtures. However, his and our methods of testing this are not exactly comparable, since he used in-culturing and we used explanting.

TABLE I

DIFFERENT NUMBERS OF BACTERIA IN SEVERAL TESTS WITH THE SAME AMOUNT OF THE SAME BLOOD. DEMONSTRATION THAT THE SAME PERCENTAGE OF ORGANISMS IS KILLED IN ALL OF THE TESTS, EVEN THOUGH, FOR EXAMPLE, MORE ORGANISMS WERE KILLED IN TEST 3 THAN WERE PRESENT ORIGINALLY IN TEST 5

DILUTION	CONTROL	2 HOURS INC.	4 HOURS INC.
1	Infinite number colonies		
2	Infinite number	Fewer	Even fewer
3	9,760	2,582 (26% of control)	208 (2.1% of control)
4	2,276	656 (28% of control)	165 (7.4% of control)
5	432	126 (29% of control)	28 (6.4% of control)

We soon found that whereas we detected differences in the bactericidal power of different bloods, duplicate set-ups with the same blood sample and the same bacterial suspension gave results almost as different as obtained with different bloods. At this stage our results were sufficiently consistent to indicate that we were experimenting in the right direction, but that our procedures needed refinement and standardization. We then investigated one possible variable after the other. We found that keeping the blood-bacterial mixtures in a warm water-bath increased the killing effect over that which occurred in the thermostat. We found that shaking the blood-bacterial mixtures at frequent intervals increased the killing effect of the blood. This caused us to install a mechanical shaking device in the warm water-bath. We found that there was less variation in duplicate samples when the blood was handled throughout in paraffin-lined, sterile containers. Replacing cotton stoppers by sterile rubber stoppers helped to give uniformity of results.

While we were in the midst of this standardization procedure, we had the good fortune to show some of our results to Dr. Karl Kassowitz. We found,

much to our pleasure, that he had been working on the problem of the bactericidal power of blood along similar lines, and was securing similar results. We had started our work independently, but he had been at work on the problem longer. He very kindly told us of an important difference between his test and ours. He was adding an appropriate number of bacteria to one sample of blood, and at hourly intervals was removing small samples for plating. He thereby obtained a sequence of results which could be charted as a curve. These curves of Dr. Kassowitz's showed that whereas some samples of blood might have the same quantitative killing effect on Staphylococcus aureus after a given interval such as two, three, or four hours, some bloods after that interval allowed the bacteria to increase in number, while others continued to kill the staphylococci for an additional period of time before the bacteria began to increase in number. It was evident that this method was an improvement on ours, and with Dr. Kassowitz's consent we adopted this modification. Dr. Kassowitz's extremely important results are reported in a paper by Drs. Gonce and Kassowitz,[6] which should be read by all interested in this field. Since the limited bibliography of this subject is discussed very completely in that paper, and since previously published methods for determining the bactericidal power of blood are criticized and reviewed, these will not be discussed here. It seems interesting that our results and our method, as finally evolved, should have paralleled so closely those of Gonce and Kassowitz. We do believe that we have paid more attention to refinements of the different procedures, and many times we have obtained duplicate results which check very accurately, almost as accurately as routine biochemical determinations. Two steps in our method seem to us to have essential and important differences from the test of Gonce and Kassowitz: one is that our blood-bacterial mixtures are shaken continuously in a warm water-bath, except for the extremely short time necessary to take samples; the other, which is possibly even more important, and which will be discussed later, is that we use heparinized blood, whereas they use defibrinated blood.

The following method is the one we now use and have used for the past year without change:

Dissolve 10 mg. of heparin in 1 c.c. of physiologic saline, and sterilize in autoclave at 10 pounds pressure for ten minutes. One mg. of heparin will keep 5 c.c. of human blood from clotting for twenty-four hours. Use approximately 0.1 c.c. of solution for each 5 c.c. of blood.

Withdraw the desired amount of blood aseptically from the patient's arm vein, and put it into a paraffin-lined glass container which holds the correct amount of heparin. Rotate gently to mix.

Centrifuge a twenty-hour broth culture of Staphylococcus aureus for three minutes at low speed to throw down large clumps of bacteria. Transfer with sterile pipette approximately 1 c.c. of the top of the centrifuged culture to a clean sterile test tube. Shake thoroughly before using. Transfer 1 loopful of shaken culture to 50 c.c. of sterile bouillon (loop should be between 3 and 4 mm. in diameter).

Melt tube of agar and cool to 50° C.

Transfer with 10 c.c. pipette 4.5 c.c. of heparinized blood into a small, paraffin-lined flask having a sterile rubber stopper (25 to 30 c.c. flasks are most easily handled).

Transfer 0.5 c.c. of the bacterial suspension to the 4.5 c.c. of blood in the small flask. Tip flask and rotate to wash down from the sides any small drops of the bacterial dilution. Rotate gently to insure even distribution of bacteria.

Transfer immediately 0.5 c.c. of the blood-bacterial mixture to the tube of melted, cooled agar. Draw agar into the pipette and blow gently out again 3 times. Stir mixture with end of pipette. Blow out gently as much as possible of blood agar remaining in pipette. Pour plate, which serves as the control.

Place flask in shaking device in warm water-bath (37° C.). Remove 0.5 c.c. at two-hour intervals for a period of eight hours and pour plates as for the control.

Incubate plates, and make accurate counts of colonies after twenty-four hours.

Results.—Only a few examples of results obtained in this investigation can be given here. Many checks and controls which were done cannot be mentioned. The data given are typical of tests repeated many times, with complete confirmation. Much of this work has been laborious and time-consuming, but we have withheld its publication until we have convinced ourselves, after a year and a half of work, of the accuracy of the results.

Although the critical study of our own procedures has been a time-consuming and complicated one, we can give assurance that the method, as finally evolved, is comparatively simple. It requires only a simple bacteriologic technic and the ordinary equipment of a bacteriologic laboratory, plus a mechanical shaker installed in a warm water-bath. We have every reason to believe that with ordinary care our results can be verified.

TABLE II

BACTERICIDAL TESTS TO SHOW ACCURACY OF METHOD. BLOOD WAS TAKEN FROM A NORMAL INDIVIDUAL AT 3 DIFFERENT TIMES DURING THE DAY, AND THE TESTS SET UP IN DUPLICATE. THE RESULTS CHECK WITHIN A FEW PER CENT

10:00 A.M.—W.B.C. 5,350

	CONTROL	2 HR.	4 HR.	6 HR.
A	3080	1344 – 43%	722 – 25.0%	Inf. number
B	3268	1224 – 37%	802 – 24.0%	Inf. number
Average	3174	1284 – 40%	802 – 24.5%	
1:30 P.M.				
A	2896	1178 – 40%	Lost	Inf. number
B	3160	1156 – 36%	796 – 20.0%	Inf. number
Average	3028	1167 – 38%		Inf. number
4:30 P.M.				
A	2912	1144 – 38%	686 – 23.0%	Inf. number
B	3184	1212 – 38%	848 – 26.0%	Inf. number
Average	3048	1178 – 38%	767 – 24.5%	Inf. number

Culture of Staphylococcus aureus eighteen hours old at 8:30. Centrifuged at low speed three minutes; 1 c.c. pipetted into clean, sterile test tube, shaken well, and allowed to stand at room temperature. From this culture, fresh suspensions were made every time the test was set up.

The accuracy of the method can be seen in the type experiment illustrated in Table II. Here blood was secured from the same individual at 10 A.M., 1:30 P.M., and 4:30 P.M. Each time the test was set up in duplicate. The widest divergence in the measure of the bactericidal power at the end of two hours is 6 per cent, in the 10 A.M. test, i.e., the difference between 37 per cent and 43 per cent. The average of these figures, however, is 40 per cent, which is a difference of only 2 per cent from the average of the results of the 1:30 P.M. and 4:30 P.M. tests. The results of the tests at the four-hour period check even closer.

It seemed important to determine whether the bactericidal power of the blood resided mainly in the cellular elements or in the plasma. We found, as

illustrated in Table III, that heparinized plasma is either devoid, or practically devoid, of any bactericidal action on Staphylococcus aureus. The same is true of serum from defibrinated blood.

TABLE III

TEST DEMONSTRATING THAT HEPARINIZED PLASMA HAS NO BACTERICIDAL ACTION ON STAPHYLOCOCCUS AUREUS, WHEREAS THE WHOLE BLOOD FROM WHICH THE PLASMA IS OBTAINED HAS MARKED BACTERICIDAL ACTION

	CONTROL	2 HR.	4 HR.	6 HR.
Whole blood – T.	492	124 – 25%	96 – 19%	368 – 74%
Plasma – T.	584	544	484	484
Whole blood – L.	444	108 – 24%	60 – 13%	62 – 14%
Plasma – L.	504	536	500	488

We next made many tests to determine, if possible, the manner in which the cellular elements of the blood removed staphylococci. This was done by removing small amounts of the blood-bacterial mixture immediately after it was made, then at frequent intervals, and later at longer intervals, making smears on glass slides and staining them with Wright's stain. With this stain both the blood cells and the bacteria stand out very clearly. Without such observations, the criticism might be made that the reduction in the number of colonies in the test is caused by the clumping of the bacteria, and not by their destruction. Table VII, we believe, demonstrates that the diminution in the number of colonies is because the cocci disappear by digestion and lysis in the leucocytes, and not because of clumping. It can be seen that in ten minutes phagocytosis has already begun, and in twenty minutes most cocci are within the polymorphonuclears. At the end of two hours practically no cocci are lying free. At four hours, when the maximum bactericidal action of the blood usually occurs in the test, there are fewer cocci present, and most of the polymorphonuclears contain only 2 or 3 cocci, instead of being loaded with them, as at the three-hour interval.

The tests of the kind just described had to be performed with the addition of a larger number of bacteria than was added when the ordinary test was performed. Ordinarily, the number of bacteria present was so small that it was difficult to find any in the small amount of blood on the smear. In some of these special tests, a comparatively large amount of a twenty-four-hour bouillon culture of staphylococcus had to be added to secure an adequate number of bacteria. In some of these special tests the leucocytes, after an hour or more, began to show degeneration and lysis, in all probability from lytic substances in the unusually large amount of added culture. Table VII represents one of the best of this group of experiments, and it was verified several times.

We next investigated whether the leucocytes decreased in number in the regular test where the usual small number of bacteria and small amount of lytic substances were added. We found, as illustrated in Table IV, by making leucocyte counts at two-hour intervals, that up to six hours there is practically no diminution in the number of leucocytes.

Because of suggestions in the article by Gonce and Kassowitz, and some of our own observations of marked bactericidal power of the blood of patients with a marked leucocytosis, we investigated the effect on the bactericidal

power of mechanically increasing the number of leucocytes in blood. This was done by securing an ample supply of heparinized blood and centrifuging most of it, removing the serum, and then sucking off with a pipette the layer of leucocytes on top of the layer of red cells. One sample of the original heparinized blood was tested, and another sample was fortified with the leucocytes removed, and tested also. The result is shown in Table V. It will be seen that the leucocyte count on the original whole blood was 10,250 per c.mm., and on the fortified blood 28,100 per c.mm., and that the bactericidal power was markedly increased in the fortified blood.

TABLE IV

DEMONSTRATION THAT LEUCOCYTES DO NOT DIMINISH APPRECIABLY IN NUMBER DURING THE PROGRESS OF THE BACTERICIDAL TEST

	CONTROL	2 HR.	4 HR.	6 HR.
White Blood Counts Whole Blood	9,550	11,750	9,200	8,000

LEUCOCYTE COUNTS WERE MADE IN THE BEGINNING AND AT TWO-HOUR INTERVALS, UP TO SIX HOURS, WHEN THE PLATES WERE POURED FOR THE BACTERICIDAL TEST GIVEN BELOW

	CONTROL	2 HR.	4 HR.	6 HR.
Whole Blood	2722	146 – 5%	410 – 15%	1760 – 63%

TABLE V

DEMONSTRATION THAT LEUCOCYTES ADDED TO WHOLE BLOOD INCREASE THE BACTERICIDAL POWER

	CONTROL	2 HR.	4 HR.	6 HR.
Whole Blood. Leuc. count – 10,250	2952	263 – 8 %	382 – 12 %	3200 – 100+%
Whole Blood plus Leuc. Leuc. count 28,100	2624	45 – 1.6%	21 – 0.8%	113 – 4.3%

Since our test was done with heparinized whole blood, and some of the methods reported, including that of Gonce and Kassowitz, used defibrinated blood, it seemed important to compare the bactericidal action of blood prepared in these two ways. It will be noted also that the results of Gonce and Kassowitz show a continuation of the bactericidal action for a longer period of time than we found with our method. The results of one of these comparisons is found in Table VI. It will be seen that the bactericidal action of the

TABLE VI

DEMONSTRATION THAT DEFIBRINATED BLOOD HAS A GREATER BACTERICIDAL POWER THAN THE SAME BLOOD KEPT FLUID WITH HEPARIN

	CONTROL	2 HR.	4 HR.	6 HR.
$Hep._1$	2796	264 – 9.4%	221 – 7.9%	3680 – 131 %
$Hep._2$	2292	190 – 8.3%	160 – 6.9%	Inf. number
Def.	2290	23 – 1.0%	14 – 0.6%	7 – 0.3%

defibrinated blood is greater than that of the heparinized blood, and continues for a longer time. The exact significance of this is not clear at present. We discovered by making blood counts that the process of defibrinating blood

caused marked reduction in the number of leucocytes. In one instance this reduction was from about 7500 to 2500 per c.mm.

It seemed of interest to determine the effect on the bactericidal power of adding plasma from defibrinated blood to cells from heparinized blood, and plasma from heparinized blood to cells from defibrinated blood. These results are shown in Table VIII. It will be noticed that here also defibrinated whole blood had a stronger bactericidal action than heparinized whole blood, and that crossing the plasma with the cells in each direction gave mixtures whose

TABLE VII

STUDY OF DISAPPEARANCE OF COCCI FROM BLOOD-BACTERIAL MIXTURE. SMEARS MADE AT THE INTERVALS INDICATED WERE STAINED WITH WRIGHT'S STAIN AND EXAMINED MICROSCOPICALLY

TIME	MICROSCOPICAL EXAMINATION
Immediately	Many cocci, the majority occurring singly; few in pairs or in 3's or 4's, but never in large clumps.
10 minutes	Cocci still very numerous. Phagocytosis beginning. Cocci grouped around and in polymorphonuclears for most part, although many cocci still occur singly.
20 minutes	Few cocci lying free or singly. Many are within polymorphonuclears.
1 hour	Many cocci phagocytized.
2 hours	Many cocci within the W. B. C. Very few lying free.
3 hours	Most polymorphonuclears loaded with cocci. Occasional one seen in a lymphocyte.
4 hours	Fewer cocci seen. Most polymorphonuclears contain 2 or 3 diplococci; occasionally a polymorphonuclear is loaded with cocci.
5 hours	Few cocci seen within the polymorphonuclears. Occasionally a cluster lies free.
6 hours	Polymorphonuclears seem to be disintegrating.
7 hours	Many cocci lying free. None of the few polymorphonuclears present are whole or healthy looking.
8 hours	(Same picture as 7.)
24 hours	Smear loaded with bacteria and débris which might be broken down W. B. C. Only an occasional lymphocyte seen intact. No polymorphonuclears.

TABLE VIII

COMPARISON OF THE BACTERICIDAL ACTION OF: 1. HEPARINIZED WHOLE BLOOD WITH DEFIBRINATED BLOOD, 2. HEPARINIZED PLASMA WITH DEFIBRINATED PLASMA, 3. HEPARINIZED PLASMA WITH CELLS FROM DEFIBRINATED BLOOD, AND 4. DEFIBRINATED PLASMA WITH CELLS FROM HEPARINIZED BLOOD

	CONTROL	2 HR.	4 HR.	6 HR.
Hep. Whole Blood	1314	162 – 12.3%	123 – 9.3%	5280 – 401%
Def. Whole Blood	1492	50 – 3.3%	6 – 0.4%	440 – 29%
Hep. Plasma	1860	1770	Inf. number	Inf. number
Def. Plasma	1890	3120	Inf. number	Inf. number
Hep. Plasma + cells from Def. Blood	1468	49 – 3.3%	22 – 1.5%	2480 – 169%
Def. Plasma + cells from Hep. Blood	1392	56 – 4.1%	42 – 3 %	920 – 66%

This test shows that:

1. Defibrinated blood under the conditions of the test has a stronger bactericidal action against Staphylococcus aureus than heparinized blood.
2. Plasma from defibrinated blood and plasma from heparinized blood show no bactericidal action.
3. Cells from defibrinated blood to which heparinized plasma has been added show approximately the same bactericidal power as defibrinated whole blood.
4. When plasma from defibrinated blood is added to cells from heparinized blood, the bactericidal action is increased to about the same as defibrinated whole blood.

If this test demonstrates anything, it is that whereas plasma from defibrinated blood has no more bactericidal action than plasma from heparinized blood, both cells and plasma from defibrinated blood, when mixed with heparinized plasma and cells from heparinized blood, respectively, increase the bactericidal action of these mixtures.

bactericidal power was approximately the same as that of defibrinated whole blood, and definitely stronger than that of heparinized whole blood. The cause of this action, which is different from what we expected, is entirely unexplained, and is well worth further study.

We have not investigated whether or not heparin has a deleterious effect on the phagocytic ability of the polymorphonuclear leucocytes. Whereas the amount of heparin used is very small, it might be that it diminishes the phagocytic ability of the leucocytes. This point, therefore, remains for further study. It is our impression, however, that the difference is not caused by the deleterious action of heparin, but by the liberation of some substance, in the process of defibrinating blood, which aids the bactericidal action. We confess that the results with defibrinated blood surprised us, as we expected that the bactericidal power would be diminished rather than increased. Defibrinated blood must injure some of the leucocytes, even though this injury is slight, and we expected this to have a deleterious effect. Whether defibrinated or heparinized blood gives more accurate results remains to be determined by further investigation. It seems to us that standardization of the technic can be more accurately accomplished by heparinizing the blood than by defibrinating it.

Practically all of our tests were done with Staphylococcus aureus. In a number of instances samples of the same blood were tested with Streptococcus hemolyticus. Not enough comparisons of this sort were made to be worth while reporting at this time. There are several results, nevertheless, of sufficient interest to mention. Although streptococci are somewhat more difficult to work with than staphylococci, the test can be made just as accurately. In the same blood the bactericidal action against these two organisms does not necessarily run parallel, and in fact is often widely divergent. Therefore, the determination of the bactericidal power of a particular blood against one pyogenic organism does not indicate its bactericidal power against any other. In the hope that parallel results would obtain, and that results with pyogenic cocci could indicate the bactericidal power against tubercle bacilli, we tested out samples of blood from several patients with pulmonary tuberculosis (secured through the kindness of Dr. Kassowitz). We found that the bactericidal power of these samples against Staphylococcus aureus and against hemolytic streptococci was quite different, and no correlation whatsoever could be made between the clinical condition or the apparent resistance of the patients to the tuberculous infection and the results of our bactericidal tests.

In the presence of a streptococcus infection the blood of several patients showed a different degree of bactericidal action against streptococci and against staphylococci, usually more against streptococci than staphylococci. In the presence of Staphylococcus aureus infections, on several occasions we found that the patient's blood had a stronger bactericidal action against his own organism than against the stock organism. In this last instance, two points are of interest: first, that the patient had a marked leucocytosis, and, second, that the staphylococcus infection persisted for quite a while in spite of a marked bactericidal power of the patient's blood. One would have expected that, in the presence of such a strong bactericidal ability of the blood, the infection would clear up rapidly. The opposite conclusion, how-

ever, seems the one indicated, that is, that even the strong bactericidal action which we found in vitro was not sufficient to eradicate rapidly the staphylococcus infection, and was probably only enough to prevent the patient from being overwhelmed by this infection.

SUMMARY AND CONCLUSIONS

A method is described for determining the bactericidal power of blood against gram positive cocci, especially Staphylococcus aureus. This method is simple and gives results which check closely in duplicate tests. The method is therefore believed to be accurate. The results obtained with it should be of relative, if not of absolute, value.

A fund is maintained by individuals and industries in Milwaukee to support Medical Research conducted in the Laboratory Department of Columbia Hospital. This work was made possible by the assistance and opportunities thus provided.

REFERENCES

1. Denison, Henry S.: Amboceptors and Complement in Typhoid Sera, Bull. Johns Hopkins Hosp. **19**: 263-268, 1908.
2. Heist, G. D., Solis-Cohen, S., and Solis-Cohen, Meyer: The Bactericidal Action of Whole Blood With a New Technique for Its Determination, J. Immunol. **3**: 261-276, 1918.
3. Matsunami, T., and Kolmer, J. A.: Relation of Meningococcidal Activity of Blood to Resistance to Virulent Meningococci, J. Immunol. **3**: 201-212, 1918.
4. Colebrook, L., and Storer, E. J.: On the Reduction of the Bactericidal Power of Blood Which is Effected by Adding to It Citrate of Soda and Other Decalcifying Agents; and on the Question Whether Blood so Treated Should be Employed for Immuno-Transfusion, Brit. J. Exper. Path. **5**: 47-54, 1924.
5. Wright, Sir A. E., Colebrook, L., and Storer, E. J.: New Principles in Therapeutic Inoculation, Lancet. **104**: 365-373, 417-420, 473-478, 1923.
6. Gonce, J. E., and Kassowitz, Karl: Action of Ultra-Violet Irradiation on the Bactericidal Property of the Blood, J. A. M. A. **90**: 280-284, 1928.

DISCUSSION

Dr. Robert G. Maul.—It is very interesting to see the increasing phagocytes in the increase of leucocytes. I would like to ask if tests were carried out with the stock leucocytic extract.

Dr. William Thalhimer (closing).—That has not been carried out.

THE INTERPRETATION OF THE WASSERMANN TEST*

By B. Markowitz, M.D., Chicago, Ill.

I SHOULD like to stress the importance of the proper interpretation which the clinician should make upon the complement-fixation test, and as illustrations I will cite some of our personal cases and references found in medical literature. Much has been written on the Wassermann test; its technic is constantly improving, and we have today almost as many varieties in its performance as we have Wassermann workers. Its interpretation, however, has been slighted; the correlation of the reaction in the tube with the existing circumstances of the specimen and its host is not so frequent a topic of discussion.

The impression made by any statement depends upon the powers of perception or interpretation of the receiver. The impression made by the Wassermann test is dependent upon the interpretation placed upon its reaction by the serologist, and the interpretation placed upon its report by the physician. These interpretations are often the basis of considerable discussion, and are sometimes the cause of different impressions made by reports on the same specimen. This, together with the small percentage of error, which must be considered, does to some degree discredit the complement-fixation test and may cause the clinician to give only half-hearted credence to the Wassermann report. For this reason, I think that with a better understanding of the interpretation of the results found, and with a better understanding between serologist and clinician, greater cooperation will be effected.

At this time, however, we will discuss only the clinician's interpretation of the serologist's report, and disregard the serologist's reading, excepting only that phase which influences the clinician's conception of the results of the Wassermann test. To do this we must first mention that even the Kolmer complement-fixation modification, considered by many the best of all Wassermann modifications, is not technically specific since we cannot explain definitely why the complement becomes fixed in a positive serum. Weygandt, as far back as 1907, and Noguchi[1] since then, proved that positive results may be obtained in syphilitic sera by using antigens made of normal tissue. Kolmer[2] states that all we know of the syphilitic reaction is that a mixture of suitable extract plus a syphilitic serum is capable of fixing large amounts of complement. Davidsohn[3] states that the positive complement-fixation test only indicates the presence of a syphilitic antibody in the blood of the patient *at the time* when the test was performed. Weil produced positive Wassermann reactions in the blood of rabbits which were injected with an alcoholic extract of rabbit organs, while Kolmer and Casselman[4] many years before showed

*Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minnesota, June 8, 9, and 11, 1928.

that the serum of many normal rabbits reacted positively to the Wassermann test. As generally performed, therefore, the Wassermann test is not a true specific reaction, and if we want the clinician to properly interpret our reports, we must not only bear this nonspecificity in mind but impress him with its existence. We must educate him to the fact that this, in addition to further information of the Wassermann test, is necessary for the proper interpretation of its reaction.

Assuming though that our reaction is a true and specific one, we still find considerable controversy arising from the variations in laboratory reports on similar cases, and the clinician is often at a loss to know how to interpret them. The report he expects is probably one to be expected, but he fails to realize that not all cases respond serologically to the accepted rule. Seven exceptions are here discussed.

1. *Sudden Serologic Change in Treated Cases.*—The sudden change in reaction of a treated case from negative to positive or vice versa is almost as well known as the chronicity of the disease itself. It frequently occurs that we report negative on a patient and two or three weeks later when the test is repeated, we find a two- or three-plus positive. To illustrate: Male, aged fifty-one, had been under treatment eighteen years ago. Three years ago he suddenly gave a four-plus reaction after constant negatives for a period of fifteen years. Since then he has been under constant treatment and observation. July, 1926, blood again became negative. Every three months a Wassermann was taken and always returned negative until September, 1927, when a two-plus Wassermann and two-plus Kahn were reported by two different laboratories. Just two weeks later, without any treatment, another Wassermann was done and this time reported completely negative by the same two laboratories. Why this sudden change occurs we do not know definitely, but as explained by Craig,[5] from these variations it is evident that the antibodies and lipo-trophic substances present in the patient's serum vary from day to day. Nichols[6] reported a case in which the patient, after two years of treatment, remained clinically and serologically negative for five years. After one injection of 0.6 gm. salvarsan, given as a provocative, his blood was negative daily for four successive days, but on the fifth day was reported four-plus positive.

2. *Serologic Variations in Untreated Cases.*—Daily titration of the blood of untreated syphilitics shows that great variations may occur in the complement binding power of the serum of patients who receive no treatment. This variation may occur from day to day so that one or even several negative examinations in a suspected case should not always be considered sufficient evidence for excluding the disease. In a series of ten cases, all suffering from undoubted syphilis in various stages, Craig[7] has shown that some cases if tested on certain days gave a negative result, although serum from the same cases had previously been positive, and again became positive in a day or two. We have found one such variation in the case of a middle-aged woman who was an undoubted luetic with a strongly positive Wassermann. She took only one injection of neosalvarsan and then disappeared for almost

a year. Upon her return her serum was reported negative by the Wassermann test, and a repetition of the test three weeks later resulted in the same report. Shortly after treatment was instituted, however, her serum again became positive. We also found a similar variation in a case not presenting any signs, or giving any history of syphilis. G. M., female, widowed, aged forty-two, one daughter aged fifteen. She had a Wassermann taken only because her roommate was found to be luetic. A history and physical examination were found negative for syphilis. The Wassermann was reported four-plus, the Kahn negative. In view of the negative Kahn, negative history and symptoms, the Wassermann was doubted and repeated one week later, at which time it was reported as a three-plus Wassermann and again a negative Kahn. Three days later the Wassermann was reported as a one-plus and still negative on the Kahn. Since then four Wassermann tests have been made on this patient and all found to be negative. We must here call attention to the importance of these results in explaining why, in sending specimens from the same patient, but at different times, to different laboratories, we find discrepancies between Wassermann reports. We must therefore bear in mind and teach the clinician that when it is desired to obtain a report from more than one laboratory upon a suspected individual, the same specimen of blood should be used; that is, obtain a sufficient quantity to be divided into two tubes and send one to each laboratory, instead of checking up at a later period on one test.

3. *Negative Wassermann in Tertiary Syphilis.*—Frequently we find a patient with a specific history of many years' standing who presents definite symptoms of the disease, and is pronounced clinically luetic. A Wassermann test is done, and, much to the surprise of the clinician, the laboratory reports a negative reaction. Two cases will illustrate: L. W., female, aged forty-nine, gave a history of definite luetic infection at the age of twenty-eight. Was then given very vigorous treatment with mercury, and for a period of two or three years took a course of treatment each year, and was pronounced cured. She presented herself to her physician with a tumor mass about the size of an orange in the left groin. He made a clinical diagnosis of sarcoma and removed a small piece which he sent to us for confirmation of his diagnosis. Tissue examinations revealed central coagulation necrosis with a peripheral zone of granulation tissue, rich in round cells; a Wassermann was suggested on the probability of this being a gumma. Despite two negative Wassermann reports which we submitted, we suggested antiluetic treatment, which we felt was justified in view of the positive history and the microscopic appearance of the tissue. This course was followed and, with the usual treatment of iodides, salvarsan, and mercury, the mass was reduced to a small nodule about the size of a walnut. After a three months' rest following treatment, the Wassermann test on this patient reacted four-plus positive. We, as serologists, must in a case like this, be able to help the clinician give the patient some reasonable explanation why, despite definite symptoms, the patient is serologically negative, and why, with treatment which reduced the symptoms, the serum reacts positive after a rest period.

The second case of this class, D. M., aged fifty-four, druggist. History of definite luetic infection at the age of thirty with subsequent treatment over a period of three or four years. Has since been free from any symptoms until several months prior to the time he presented himself for examination, when he noticed that his memory was failing. His physician elicited definite luetic symptoms and sent him in for a Wassermann test which was negative on three different occasions. With a definite history and Argyll-Robertson pupils, we suggested a spinal fluid examination on which was returned a negative report on the Wassermann test, but a cell count of 34, globulin strongly positive, and a tabetic reaction in the Lange colloidal gold curve. Despite the negative Wassermann a diagnosis of tabes was made, and the patient improved under antiluetic treatment. Following a two months' course of treatment the patient was given a rest of six weeks, at which time his spinal fluid was four-plus positive, and the patient felt well.

Solomon and Klauder[8] report similar findings of negative spinal fluid Wassermanns in known luetics, particularly in cases with vascular lesions. Wynn[9] reports the disappearance of spinal fluid findings in cases of paresis, although symptoms continued.

According to the statistics of Lindlan, only about 60 to 75 per cent of the cases of tertiary syphilis gave positive results. In general paresis he reports the blood positive in 82 per cent of the cases, and the spinal fluid positive in practically 100 per cent. Boas (quoted from[1]) reported that in early latent syphilis 40 per cent were found with positive reactions; in old latent cases he reported 22 per cent of properly treated cases and 74 per cent of insufficiently treated cases, as positive.

As shown by these cases, the physician must not immediately convict the laboratory of poor work if the Wassermann report does not compare favorably with the clinical symptoms, but must bear in mind that tertiary syphilis will sometimes fail to give a positive Wassermann reaction.

4. *Mild Positive Wassermanns in Certain Nonluetic Individuals.*—On the other hand the reverse condition is not infrequently found; a mild positive Wassermann in a nonluetic, as for instance in a case sent us: B. W., painter, middle-aged adult male with a score of chronic complaints, but no definite train of symptoms. No specific history of any kind was given, and on examination there were no physical findings indicating a specific infection. His mouth was in poor condition, teeth were carious, and there was a considerable degree of pyorrhea. As a routine matter a Wassermann was done and returned from the laboratory as a mild positive. The Wassermann was twice repeated and both times reported as a two-plus positive. We suggested that the teeth and gums be treated. Several abscessed teeth were extracted, and prophylactic treatment instituted. Six months later the patient's physical condition was greatly improved and the Wassermann test returned negative on two different occasions. This false reaction is not infrequently found in low grade systemic infections as illustrated. The careful internist will not, in this case, make a diagnosis of syphilis on the strength of the Wassermann

alone, but will place the proper interpretation on the report submitted, and together with the history and physical findings, make a diagnosis other than syphilis.

The pathologic laboratories of the Mount Sinai Hospital at Philadelphia reports two cases in which weakly positive reactions were found in jaundiced patients. Corrigan[10] reported, on serum from 100 febrile patients, two strongly positive Wassermann reactions in cases of pneumonia with no syphilitic histories, and in one of these the Wassermann became negative after the crisis. One strongly positive reaction was found in a nonluetic case of endocarditis, and a weakly positive reaction was found in a case of epilepsy.

5. *Wassermann Fast Cases.*—Another condition in which the Wassermann report does not compare favorably with the clinical course, is the so-called Wassermann fast cases, in which the Wassermann reaction is persistently 100 per cent positive despite the repetition of vigorous courses of antiluetic treatment. I am mentioning the following case only because it differs slightly from the usual Wassermann fast case in that the serum of this patient became negative after the first course of treatment, and later again became and persistently remained strongly positive. Patient S. D., salesman, male, aged nineteen, presented himself with definite secondaries and a four-plus Wassermann in 1921. Following vigorous course of treatment, consisting of eighteen intravenous injections of neosalvarsan and forty intramuscular injections of mercury, one grain each, all given within a period of three months, his Wassermann came down to a one-plus after two months rest. Instead of reporting for another course of treatment as he was instructed, this patient failed to return. A year later he returned and again his Wassermann was reported four-plus. Since then he has been given many courses of treatment, including various preparations of salvarsan, mercury, and bismuth, but his blood was persistently four-plus positive. He has had to date over 100 intravenous injections of salvarsan, and about twice as many intramuscular injections of mercury and bismuth, with no serologic change.

Beinhauer and Jacob[11] think that in some cases the "Wassermann fast" syphilis is due to tissue saturation by the heavy metals used in the treatment. In this case, however, we showed serologic progress after a very vigorous course of treatment, but after neglecting treatment for a year the serum became and remained strongly positive.

6. *Negative Wassermann in Primary Stage.*—A negative Wassermann reaction, in the primary stage of syphilis is of no value whatever in eliminating the disease. Even if this negative reaction persists until the initial lesion disappears and during the stage preceding the onset of the secondary symptoms, it cannot be relied upon as evidence that syphilis does not exist, because the Wassermann test is frequently negative in the presence of an undoubted hard chancre. Bruck did report very early positive reactions in monkeys, even before the appearance of the lesion, but we are still to face the problem that some are negative long after the appearance of the lesion. Klauder and Kolmer[12] reported positive reactions in the secretion of the primary lesion, while the blood serum reacted negatively. According to statistics of Craig[7] reporting only strongly positive reactions, complete in-

hibition of hemolysis, in the first week after the appearance of the initial lesion, 14 per cent are positive, in the second week 22 per cent, in the third week 41 per cent and in the fourth week 53 per cent.

Rosenau[13] states that the Wassermann reaction becomes positive by the tenth day in about 30 per cent of the cases, although it has occasionally been noted as early as three days after the initial lesion appeared. It is positive in 70 per cent by the end of the third week, and in 96 per cent of all cases by the fortieth day. That still leaves us a very large number of negatives in known positive cases. In our series of 42 cases, with no record of the age of the lesion, we found positive Wassermanns in only 24, or about 59 per cent.

7. *Wassermann Test Influenced by Ingestion of Alcohol.*—That the ingestion of alcohol greatly influences the Wassermann reaction was definitely shown by Craig and Nichols,[14] who demonstrated by a series of nine cases that the positive reaction disappeared after its administration, and again reappeared after periods varying from several hours to several days. In these cases the blood was tested one hour before alcohol administration was begun, and one hour to several days after the last dose of alcoholic liquor had been administered.

In conclusion I would like to emphasize the following salient points:

1. The Wassermann test per se is not a truly specific test for syphilis, but rather an accidental finding; some, as yet unknown, biochemical change is present in the luetic individual, which causes the complement to become fixed.

2. Variations occur in the Wassermann reaction, depending upon the stage of the disease, early or late treatment, and individual influences produced by the host.

3. We must educate physicians to use the Wassermann test not as the main diagnostic factor in syphilis, but rather as one of the findings or symptoms of the case.

4. The proper interpretation of the report submitted by the serologist is the important factor in using the Wassermann test as an aid to diagnosis.

5. This interpretation, when properly understood, will effect greater cooperation between serologist and clinician.

REFERENCES

1. Noguchi, H.: The Relation of Protein, Lipoids and Salts to the Wassermann Reaction, J. Exper. Med. **11**: 84-99, 1909.
2. Kolmer, J. A.: A Practical Textbook of Infection, Immunity and Specific Therapy, Philadelphia and London, W. B. Saunders, pp. 899, 1915.
3. Davidsohn, I.: The Laboratory Diagnosis of Syphilis, Am. J. Syph. **12**: 122-135, 1928.
4. Kolmer, J. A., and Casselman, A. J.: Concerning the Wassermann Reaction With Normal Rabbit Serum, J. Med. Res. **28**: 369-375, 1913.
5. Craig, C. F.: The Wassermann Test, St. Louis, C. V. Mosby Co., pp. 239, 1918.
6. Nichols, H. J.: An Analysis of Anti-Typhoid Vaccines and a Consideration of Their Relative Practical Value at the Present Time, War Dept. (U. S.) S. G. O., Bull. No. 2, Wash., 23-36.
7. Craig, C. F.: Variations in the Strength of the Wassermann Reaction in Untreated Syphilitic Infections, J. A. M. A. **62**: 1232-1236, 1914.
8. Solomon, H. A., and Klauder, J. V.: Neurosyphilis With Negative Spinal Fluid, J. A. M. A. **77**: 1701-1706, 1921.

9. Wynn, J.: Paresis With Negative Spinal Fluid, Am. J. Syph. 7: 323-325, 1923.
10. Corrigan, N.: Nonspecific Wassermann and Agglutinin Reactions With Serums From Patients With Febrile Diseases, J. Infect. Dis. 41: 457-460, 1927.
11. Beinhauer, L. G., and Jacob, F. M.: Sodium Thiosulphate in Wassermann-Fast Syphilis, Am. J. Syph. 12: 61-66, 1928.
12. Klauder, J. V., and Kolmer, J. A.: The Wassermann Test Performed With Chancre Fluid as an Aid to the Early Diagnosis of Syphilis, Arch. Dermat. & Syph. 5: 566-569, 1922.
13. Rosenau, M. J.: Preventive Medicine and Hygiene, New York and London, D. Appleton & Co., ed. 5, pp. 1458, 1927.
14. Craig, C. F., and Nichols, H. J.: The Effect of the Ingestion of Alcohol on the Result of the Complement Fixation Test in Syphilis; a Preliminary Note, J. A. M. A. 57: 474-476, 1911.

PRESERVATION OF COMPLEMENT, A PRACTICAL STUDY*

By B. W. Rhamy, M.D., Fort Wayne, Indiana

IN 1917 I[1] suggested the use of sodium acetate as a preservative of complement, setting forth that it would preserve complement a reasonable length of time, that it was not anticomplementary even in saturated solution, and that such preserved complement could be diluted with salt solution to any desired strength.

In Kolmer's[2] classical study of the Wassermann test he compared several methods of preserving complement including sodium acetate. He concluded that 17 per cent sodium chloride yielded the best results. By this method 17 per cent sodium chloride was added to full strength guinea pig serum. For use 19 c.c. of distilled water were added to 1 c.c. of serum making a 1-20 dilution in isotonic salt solution. While I have adopted the Kolmer Wassermann technic with enthusiasm, I was loath to discard the sodium acetate method of complement preservation, without first satisfying myself of the material superiority of salted complement. I therefore ran a parallel series, using both preparations of complements, over a period of three years on all Wassermanns coming into the laboratory. Pools were made from the total blood of four or five guinea pigs and the serum divided into two portions. To portion No. 1 was added sodium chloride to make a 17 per cent mixture. To portion No. 2 was added 6 parts of 12 per cent sodium acetate solution to each 4 parts of serum, making a 40 per cent stock complement dilution containing 8 per cent of sodium acetate. This was placed in 5 c.c. vials and kept in the same compartment in the ice box with the salted complement.

These preserved complements Nos. 1 and 2 were then used in the same dilution (1-20) in making the daily Wassermann tests but on alternate days, except that on days when unusual numbers of Wassermanns were run, then the other complement was used on two successive days to keep the amounts equal, in order that one should not be exhausted before the other. Both complements were titrated the first day and after that on the days used. When either showed loss of fixability or tendency to false positives they were dis-

*Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minnesota, June 8, 9, and 11, 1928.

carded. Ox and human blood cells were used at different times, preserved with 0.125 per cent formalin. In this study, sharp changes in the unit strength of both complements will be noted, due to variations in the resistance (fragility) of different batches of red blood cells, as I had previously pointed out. The variations noted are mostly of lowered resistance of new batches of cells, since it was not practical, except in two instances, to distinguish whether an increase of amount of complement needed was due to loss of complement strength or to increased cell resistance. Considerable variation was noted in the unit strength from pigs obtained from different sources. One source of supply furnished pigs whose serum was weak in complement. Changing the source of supply resulted in getting a much better quality of complement.

In this study the main consideration was not so much how long complement was preserved, but rather the comparative behavior of the two forms of preserved complement made up from the same serum. For this reason the fact that the charts show samples of extremely poor complement, as well as good at all seasons of the year, makes the study all the more complete.

The number of days any one lot of preserved complement was in use depended on the following factors: First, the total quantity of any one lot of complement and its titer. Second, the volume of Wassermann tests to be done. Third, weather conditions: i.e.,

a. Season of the year (complement keeps best in cold weather).
b. Sudden sharp variations in barometric pressure sometimes caused sudden marked deterioration of preserved complement.

In charting this study I divided the series into the four seasons of the year, to show the effect of weather.

SERIES FOR THREE SPRINGS

The poorest lot of complement was good for seven days, the best for twenty-eight days, and the second best twenty-four days. Of 12 lots of complement used during the spring months, the average period of serviceability was sixteen days. In this group the units of both complements ran parallel with one exception. In the second lot the salted complement jumped from 0.35 c.c. to 0.45 c.c. on the eleventh day, then to 0.6 c.c. while the acetated complement did not go above 0.4 c.c. There were seven instances where new batches of cells had lower resistance than the previous batch, and therefore required less complement. In this group the first drop in complement strength averaged at two and one-half days, the second drop at nine days.

SERIES FOR THREE SUMMERS

During the hot months both forms of complement deteriorated more rapidly and required for the three summers 19 lots of serum. Two lots were discarded after four days, while the best lot was in use nineteen days. The average period of use was ten days, the first drop in strength averaging at two days, the second at five days. In this group, the salted complement deteriorated more rapidly in one instance and the acetated complement in one instance, so in the entire group the honors were even. In six instances new

cells with lower resistance required less complement. In one instance, the third lot, new cells on the tenth day were of much greater resistance requiring twice as much complement.

SERIES FOR THREE FALLS

In this group showing nine lots of complement the poorest lot lasted seven days, the best twenty-one days. The average period of use was fifteen days. The first drop averaged at three days, the second at eleven days. The salted complement deteriorated most rapidly in three instances, as against acetated complement once. Otherwise both complements ran parallel in quality. There were three instances where new cells of lower resistance required less complement.

SERIES FOR THREE WINTERS

In this group 11 lots of complement were used. Two lots lasted only seven days, while the best lot was used for thirty-one days, beginning with a unit of 0.1 c.c. and finishing at 0.3 c.c. The average period of use was nineteen days. There were nine instances where new cells of low resistance required less complement and one instance (the first lot of the group) where the opposite is very definitely shown. The unit on the first day was 0.75 c.c. but on the seventh day with new cells only 0.15 c.c. was required, finishing on the nineteenth day at 0.2 c.c., proving it to be an excellent lot of complement. Both complements ran parallel in quality with two exceptions. In both instances it was the acetated complement that went bad. In this group there was no regularity of the first drop in complement strength. In seven lots it averaged on the third day while in four lots of exceptionally good complement it averaged at eleven days. The second drop averaged at eleven days. In the last lot there was no second drop of unit strength.

SUMMARY

A summary of these experiments indicates that during the spring months both complements were used from seven to twenty-eight days, averaging fifteen days, during the summer months four to sixteen days, averaging ten days, during the fall seven to twenty-one days, averaging fifteen days, and during the winter seven to thirty-one days, averaging nineteen days.

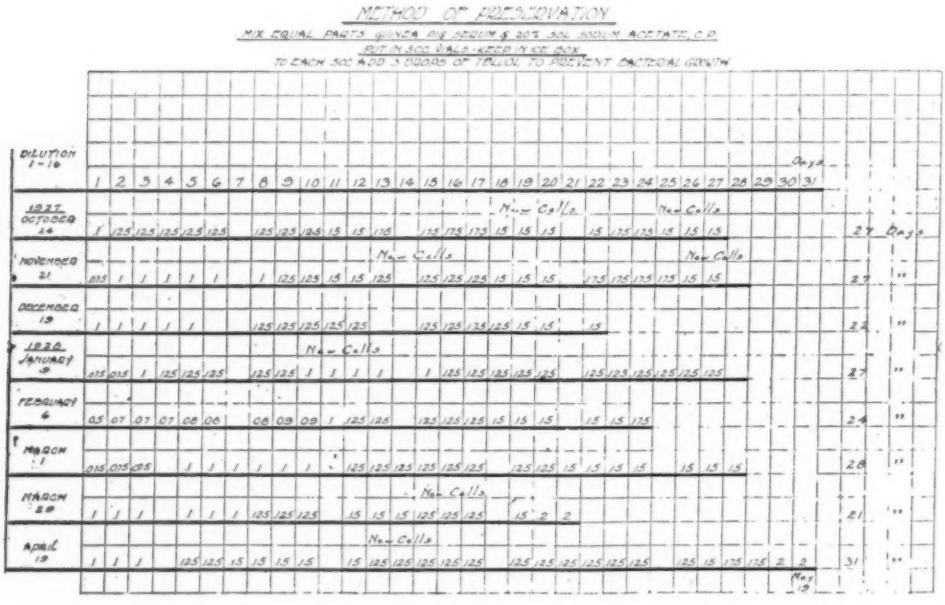
There were 25 instances where the use of new batches of cells required a smaller complement unit, indicating that they had less resistance to hemolysis (greater fragility) than the previous batches. In two instances it was possible to note that new batches of cells showed definitely higher resistance to hemolysis. The first drop of complement strength ordinarily occurred on the second or third day, the second drop around the tenth day. A few lots of complement registered only the initial drop. Adult pigs furnished the best complement.

There were five instances in the whole series where the salted complement proved inferior and four instances where the acetated complement proved inferior. With this slight difference, neither form of complement proved materially superior to the other, unless the following propositions be considered exceptions:

1. (a) Salted complement is capable of only one dilution, and if it be weak, large amounts must be titrated. (b) Salted complement after dilution for use is subject to the same deterioration that may take place in unpreserved complement, when out of the ice box for titration and during the various steps of the Wassermann technic (important in hot weather).

2. (a) Because sodium acetate is not anticomplementary even in saturated solution (63 per cent) this form of complement can be used in any strength or dilution desirable, and on account of this flexibility of dilution the operator can regulate the amount to be pipetted. (b) No matter what the dilution of acetated complement, the sodium acetate is still present acting as a preservative. For example, a 1-16 dilution of 50 per cent stock dilution (equal parts

TABLE I



of serum and 20 per cent sodium acetate solution) making approximately 1-32 serum dilution would contain $\frac{5}{8}$ of 1 per cent sodium acetate. Even this small amount will prevent deterioration while the complement is out of the ice box. Closer titration is therefore possible.

As conclusions I might indicate that,

1. In my own experience there is no practical difference in these two forms of preserved complement as to stability, fixability or tendency to give false positives.

2. Salted complement requires accuracy in weighing and dilution and after dilution for use is subject to the same deteriorations common to unpreserved complement.

3. Complement preserved by sodium acetate in my opinion has three qualities to commend it:

1. Flexibility of dilution.
2. Ease and simplicity of preparation.
3. Retention of its preserving properties in any dilution, allowing closer titration.

Having justified my faith in acetated complement, my next aim was to improve it. Sodium acetate has no bactericidal properties, 10 per cent solutions having no inhibitory action on the growth of streptococci. I have found that the temperature of the ice box depended on the uncertainties of the ice man. Sometimes it was full of ice but often nearly empty and occasionally entirely so, especially on a few very hot Sundays and holidays. At these times acetated complement was subject to deterioration from bacterial contamination. To prevent this I began adding three drops of toluol to each 5 c.c. of acetated complement. I have also increased the strength of the sodium acetate solution to 20 per cent and now make a 50 per cent stock complement dilution. Then about a year ago I got rid of the ice man problem by installing an ice machine. With this constant temperature plus the toluol and 20 per cent acetate solution the limits of usefulness of a lot of acetated complement is now apparently only limited by the total quantity at hand. As shown in Table I whatever amount was made up was used in its entirety, lasting on an average of about four weeks. The unit of complement at no time shows any wide variation from the first day's titration, averaging in the end about a half more. One lot of complement shown in Table II was made up Aug. 4, 1927, and lasted until Sept. 23, 1927, a period of fifty-two

TABLE II

COMPLEMENT TITRATION BEGINNING AUGUST 4, 1927

DAYS	AMOUNT IN MILS	DAYS	AMOUNT IN MILS
1*	0.15	27†	0.1
2*	0.15	28	0.1
3*	0.15	29	0.1
4	——	30	0.125
5*	0.2	31	0.125
6*	0.2	32	——
7*	0.2	33	0.15
8*	0.2	34	0.15
9*	0.2	35†	0.125
10*	0.2	36	0.125
11	—	37	0.125
12*	0.2	38	0.125
13*	0.2	39	——
14*	0.2	40	0.125
15*	0.2	41	0.125
16	0.125	42	0.125
17	0.125	43	0.15
18	——	44	0.15
19	0.125	45	0.15
20	0.125	46	——
21	0.125	47	0.15
22	0.125	48	0.15
23	0.125	49	0.15
24	0.125	50	0.15
25	——	51	0.15
26	0.125	52	0.15‡

*Dilution 1:32, all other titrations done with 1:16 dilution.
†New cells.
‡All of this batch used up September 24th.

days, giving sharp reactions to the end. The first dilution was 1-32, later 1-16. The unit on the first day was 0.15 c.c. (dilution: 1-32), and on the fifty-second day it was just double or 0.15 c.c. of a 1-16 dilution.

REFERENCES

1. Rhamy, B. W.: Preservation of Complement, J. A. M. A. 69: 973-974, 1917.
2. Kolmer, J. A., Matsunami, T., and Trist, Mary E.: Studies in the Standardization of the Wassermann Reaction. V. The Preservation of Complement Serum, Am. J. Syph. 3: 513-540, 1919.

DISCUSSION OF PAPERS BY MARKOWITZ AND RHAMY

Dr. H. C. Sweany.—Regarding the complement-fixation reaction in syphilis, we have made a few observations in our tuberculosis patients in Chicago, realizing that this reaction in certain cases is quite fleeting, so much so that before we permit the treatment of one of our tuberculosis patients we get two or three positive reactions on this patient before we send in the report. Now it seems to me that we must learn more of the physicochemical reaction taking place in the Wassermann before we should put too much stress on these borderline cases. It may be illustrated very well by what happens in tuberculosis. In this disease we have made an extensive study of the blood chemistry changes. We find that the albumin-globulin ratio varies many times quite abruptly in a very short period of time, so that certain physicochemical reactions taken from week to week will vary a good deal. It seems quite reasonable therefore, that the same thing may happen in syphilis except there is a definite relation there of the proteins to the disease. Until we know more of the chemistry of this reaction we must be careful in our evaluation of this test. Clinicians must also be taught to interpret the laboratory man's report.

Dr. J. J. Moore.—Dr. Markowitz's paper is very important. It is very disconcerting for a clinician to send separate samples of the same blood to three laboratories approved by the American Medical Association, directed by members of this Society and get three different reports. It seems to me that we have not gone far enough in our cooperation with each other in seeing that our reports agree. In the last year in Chicago our laboratory and another approved laboratory have run a number of Wassermanns upon the same individuals with no agreement. Fortunately for us since our reactions were positive, all these cases were old treated lueties. Another approved laboratory has agreed consistently with us for the last three years. We have now suggested that those directors of approved laboratories in the same localities get together and find out what is wrong with their Wassermanns. Many clinicians at the present time get up and say the Wassermann is of no value whatever. I would not take any four-plus Wassermann as a diagnosis of syphilis unless it was backed up by other reports or clinical symptoms. We can get together and get our antigens so fixed up that the laboratories in the same commuunity can agree more closely on bloods taken on the same patient and sent to different laboratories.

Dr. Wm. G. Exton.—Some of you will remember that in the symposium we had at San Francisco this same topic was thoroughly discussed, and that I brought to your attention the fact that insurance offices are constantly puzzled by the discrepancies reported from the laboratories in the different parts of the country which do Wassermann tests. I think that the crux of the situation to some extent at least, lies in the fact that not only are different methods used but that when they do the same test, such as Kolmer's technic, each has some different little quirk that may bring its work out of line with the others. Personally, I regard the whole matter as a positive scandal which is going to hinder clinical pathology if some way is not found to get uniform results from different laboratories.

Dr. Frederic E. Sondern.—Dr. Moore's and Dr. Exton's statements are well founded. They are brought about by various conditions as the reader of the first paper indicated in closing. Communications of this kind are of value chiefly to the clinician. We are apt to talk about these things among ourselves but often fail to go before the clinician's societies and talk in the same way, which is our duty as the clinician is often not sufficiently informed on the very things that we are now considering. The one great difficulty is the variation

in reaction of people that have been treated for syphilis. Dr. Markowitz spoke of several conditions in which a patient was negative one day and positive later on and negative again. It brought to my mind one instance that I experienced. In this case my reaction was completely negative and that of another laboratory was completely positive. The blood for these tests was not taken at the same time but on the same day. They were repeated one week later and this time my test was completely positive while the other was completely negative. Subsequent investigation proved that the two negatives were taken in the afternoon and the two positives in the morning, and we also found that it was the patient's habit to drink three high balls at noon. If Dr. Markowitz's paper were read to a society of clinicians I think it would do a great deal of good.

The question of nonspecific positives is also important. The seriousness of a false positive reaction affects the private laboratory worker to a greater extent than the serologist of a large institution. In the latter the tests are more frequently repeated and the error is generally discovered more quickly. If the result of the Wassermann test is used conjointly with clinical manifestations in diagnosis and prognosis harmony generally results, but if the result of the Wassermann test is the only factor used for this purpose and particularly if the patient is taught to follow his own case on the basis of Wassermann reports, only then it is not unusual to have a confused clinician and a doubting patient.

Dr. A. H. Schade.—Dr. Sondern has hit the nail on the head. In Toledo the pathologists attempt to get a paper before the academy of medicine at least once a year on the interpretation of the Wassermann test, stressing that the Wassermann test is only one of the symptoms of syphilis.

Dr. B. Markowitz.—I have not much to add except that as Dr. Sondern has pointed out it is very important to educate the physician on this point of Wassermann reaction. I think we should take it upon ourselves as Dr. Moore says, that every one of us should go back to his locality and get together with other pathologists in the community and decide upon a technic that would come at least close to being the same. We should attempt, in that particular community before the different medical societies, to point out that idea of false positives in syphilis.

Dr. A. H. Sanford.—It seems to me that Dr. Rhamy's paper should have some discussion. Just one question; he talked about using new cells, I am not quite clear as to what he uses. Do you use pooled cells from the slaughterhouse? Do you take one sheep and bleed him or do you use a number of sheep?

Dr. B. W. Rhamy.—I used both ox and human cells preserved in 0.125 per cent formalin. When human cells were used a pool was made from whatever fresh cells were available in the samples at hand for Wassermann tests. These suspensions were used for about three days, then a new supply made up.

Dr. A. H. Sanford.—The reason I asked was that we have had the experience that when we used one sheep right along, the cells will finally become resistant from that sheep so that we have to get a new one. We find that there is quite a little variation in sheep cells when we start in using new sheep.

Dr. B. W. Rhamy.—I had one batch of complement, whose unit on the first day it was titered, was 0.75 c.c. of a 1-16 dilution. Four days later with a new batch of cells it was 0.15 c.c. The cells that were used the first day were evidently of extremely high resistance. They were ox cells and were hard to separate from the serum.

THE SEDIMENTATION TIME OF BLOOD IN JAUNDICE*

By N. Rosenthal, M.D., and M. I. Blowstein, M.D., New York

JAUNDICE is a symptom which may result from numerous factors; these are sometimes obvious, although often obscure. The diagnosis of certain forms of hemolytic jaundice, such as pernicious anemia and congenital, splenomegalic, acholuric jaundice, can readily be made from the blood picture and the fragility of red blood cells. In cases of cholelithiasis, new-growths, catarrhal jaundice and cirrhosis of the liver accompanied by jaundice, however, the problem is more difficult; no definite laboratory finding being considered diagnostic in these conditions. Nevertheless, the intensity of the jaundice may be determined by the icterus index; the possible extent of liver damage may be ascertained in cases of nonobstructive jaundice by the bromsulphthalein test (S. Rosenthal[1]), and the degree of obstruction in bile ducts or liver cells may be estimated from examinations of the urine and stools for urobilin, bile and color. Complete obstruction of the bile ducts or in the liver cells is shown by the absence of urobilin in the stools and urine, and by the passage of clay-colored stools. Again, the presence of leucin and tyrosin in the urine are to be regarded as important findings, being indicative of liver degeneration.

Recently the sedimentation reaction of red blood cells was proposed as an additional aid in the differential diagnosis of certain cases of jaundice. As the conclusions reported by Katz and Leffkowitz[2] concerning the sedimentation rate in jaundice were based on comparatively few cases, it was believed that a more extensive and systematic study of this reaction might more fully determine its extent or limitation. With this purpose in mind, observations were made by us in a series of 69 cases, in which a definite diagnosis was made by operation, x-ray, autopsy, or clinical observation, especially in cases of catarrhal jaundice and cirrhosis of the liver.

METHODS EMPLOYED

The Linzenmeier[3] method for the sedimentation rate was employed in the present series. For this purpose citrated blood was used in a proportion of one part of 5 per cent sodium citrate to four parts of blood. The sedimentation time in the usual Linzenmeier tubes was noted when the red blood cells reach the 18 mm. mark. The normal time with this method was 180 minutes or longer. For the determination of the icterus index, we used the Brown[4] modification of the Meulengracht[5] reaction. Our experiences were similar to those of Shattuck, Killian, and Preston,[6] regarding the dependability of the icterus index. The direct reading of the color of the serum or plasma was more sensitive than the Van den Bergh reaction in milder grades of jaundice.

*From the Medical Department and Laboratories, Mt. Sinai Hospital, New York.

*Read before the Seventh Annual Convention of the American Society of Clinical Pathologists; Minneapolis, Minn. June 8, 9, and 11, 1928.

EFFECT OF JAUNDICE ON THE SEDIMENTATION RATE (CHART 1)

About 80 per cent of our cases accompanied by jaundice showed a sedimentation time which was less than normal. Most of these cases were rapid, between eleven and eighty minutes. The sedimentation rate bears no relation to the degree or duration of jaundice or of bile obstruction. These results seem to be rather paradoxical. According to the experiments in vitro by Johannes and Joseph Vorschütz,[7] and by Katz and Radt,[8] bilirubin itself does not influence the sedimentation rate, but bile salts have a marked inhibitory effect. Cholesterol, according to Kürten[9] hastens, and lecithin inhibits the sedimentation rate of the red cells. The fibrinogen and globulin of the blood, in jaundice, as reported by Starlinger,[10] seem to be increased except in grave icterus, where the fibrinogen is greatly diminished, as pre-

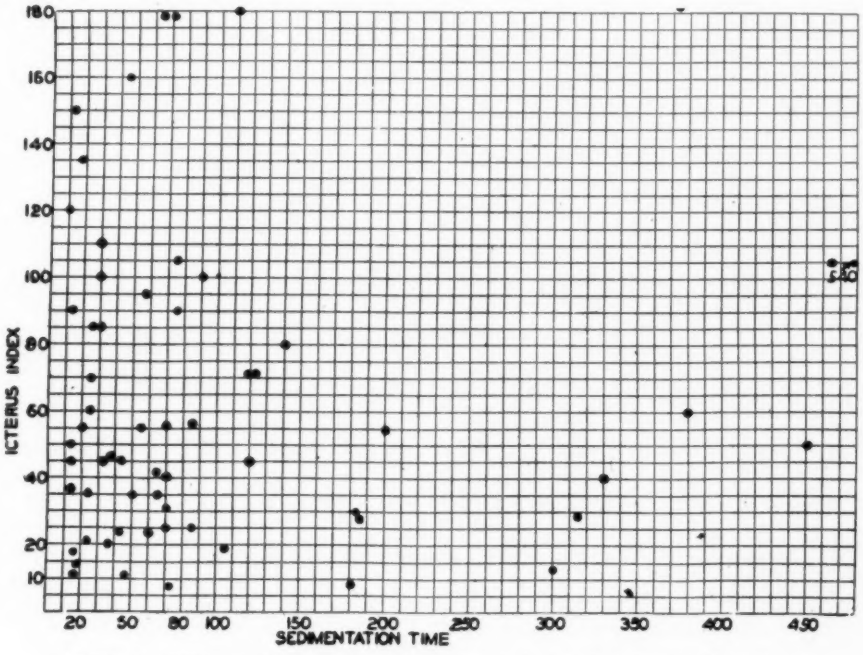


Chart 1.—Corresponding values of icterus index and sedimentation time.

viously shown by Whipple,[11] Gram,[12] and others. Synergistic and antagonistic elements which affect the sedimentation rate are therefore present in icteric plasma. The elements which inhibit the sedimentation of the red cells, as the bile salts, are increased in practically all cases of jaundice, according to the observations of F. Rosenthal and Wislicki,[13] and Shattuck, Katayama, and Killian[14]; and the lipin phosphorus (lecithin) is increased in obstructive jaundice (Byrom and Kay[15]). In spite of the presence of these inhibitory factors in icteric plasma the sedimentation rate in most cases of jaundice shows a marked tendency to increase. Fibrinogen and globulin may influence but do not show any constant correspondence to the sedimentation rate, as found by Fahraeus[16] and later by Pinner,[17] conclusions, however, which do not agree with those reached by Gram,[18] Frisch and Starlinger,[19] on the relation of

fibrinogen to the sedimentation rate. The effect of jaundice on the dispersion state of globulin which is believed to be the main factor in the sedimentation of the red blood cells, is problematical.

Johannes and Joseph Vorschütz[7] state that jaundice of short duration does not affect the sedimentation rate, but that jaundice of long standing slows the rate. They attribute this action to the increase of bile salts and lecithin. Katz and Radt[8] confirm the observation of the effect of bile salts in vitro and believe that the retention of bile salts affects the sedimentation rate in cases of jaundice. On the other hand, Löhr[20] found a rapid sedimentation time in cholelithiasis with or without jaundice; but in such cases Shattuck, Katayama, and Killian[14] have shown a marked retention of bile salts in the blood.

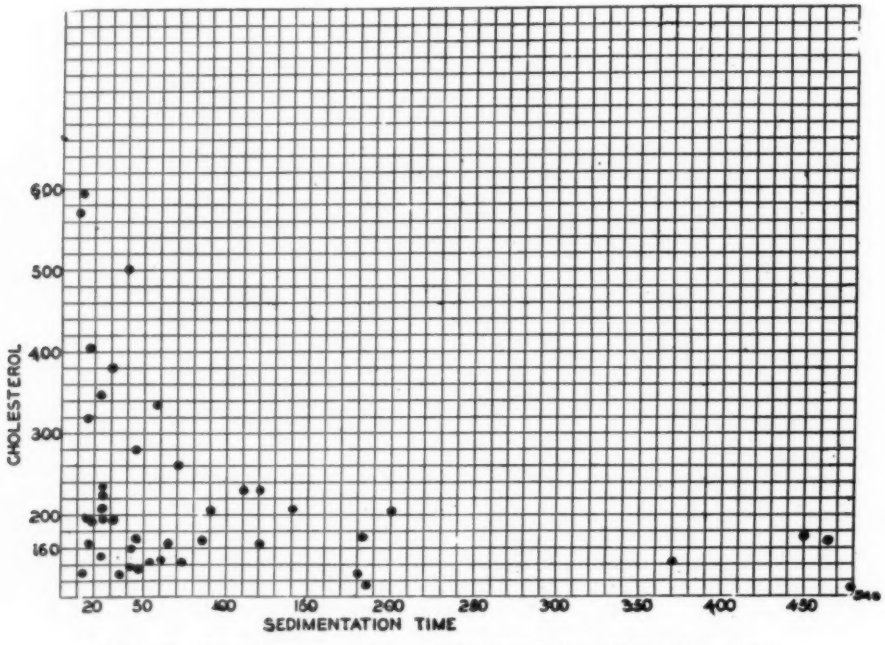


Chart 2.—Corresponding values of cholesterol and sedimentation time.

Another important factor in hastening the sedimentation rate is the presence of an infection. Most of the cases here reported, however, were afebrile; so that the increased sedimentation rate in our cases may be attributed to changes in the icteric plasma, which are not yet clearly understood.

RELATION OF CHOLESTEROL TO THE SEDIMENTATION RATE (CHART 2)

Kürten[9] has shown that cholesterol increased the sedimentation rate of the red blood cells. Pinner[17] found no definite relation between the cholesterol and the sedimentation rate in cases of tuberculosis. We, however, note a definitely rapid sedimentation rate of the blood when the cholesterol is greater than 300 mg. In two cases of disturbed cholesterol metabolism with hypercholesterolemia (500-800 mg.) without jaundice, the sedimentation rate was also

found to be rapid. It would thus appear that a greatly increased cholesterol of the blood is an important factor in the sedimentation of red cells in cases of jaundice.

SEDIMENTATION RATE IN VARIOUS DISEASES ASSOCIATED WITH JAUNDICE

1. *Carcinoma* (Table I).—In a series of 18 cases of newgrowth accompanied by complete or nonobstructive jaundice, proved by autopsy, x-ray or operation, the sedimentation rate was rapid in all except two cases. In one case, the sedimentation time was normal (185 minutes and, later, 315 minutes). This patient presented a moderate degree of jaundice (icterus index 28) and a definite polycythemia which was responsible for the slow sedimentation rate. The

TABLE I

CARCINOMA

CASE	S.R.	L.F.T. %	I.I.	CHOL.	COMMON DUCT OBSTRUCTION	REMARKS
1. K.H.	11	100	150		Complete	
2. S.B.	18	25	12	182 mg.	None	
3. H.H.	25	70	70	224 mg.	Complete	Ca. Pancreas
4. S.B.	25	60	60	248 mg.	Partial	Anemia
5. I.G.	25	35	90		Complete	Metastatic Ca.
6. F.S.	30	60	85	380 mg.	Complete	Sarcoma of Intestines
7. S.B.	30	100	110	230 mg.	Complete	Ca. of Common Duct
8. M.P.	30	70	100	182 mg.	Complete	Ca. Papilla
9. A.S.	33	40	45	216 mg.	Complete	Ca. Stomach
10. E.T.	35	25	30		None	Metastatic Ca.
	25	35	18		None	
11. H.M.	38	40	46	130 mg.	None	Metastatic Ca. of Liver
12. B.G.	58	40	95	340 mg.	Complete	Ca. Pancreas
13. D.G.	70	70	45		Complete	Ca. Pancreas, Anemia
14. I.G.	85	85	56	176 mg.	Complete	Ca. Common Duct
15. M.S.	110		180	230 mg.	Complete	Ca. Pancreas
16. S.A.	120	70	45	164 mg.	None	Metastatic Ca. of Liver
17. J.S.	185	50	28	124 mg.	None	Hypernephroma
	315	35	28			Thrombosis of Hepatic veins. Polycythemia
18. F.A.	360		15	160 mg.	None	Metastatic Ca.

S.R.=Sedimentation rate-minutes.
L.F.T.=Liver function test.
I.I.=Icteric index.
Chol.=Cholesterol.

postmortem findings showed a hypernephroma of the kidney, with involvement of the vena cava and thrombosis of the hepatic veins. No metastases were present in the liver. Blood concentration in the other case of carcinoma and jaundice probably influenced the sedimentation rate. All patients were afebrile, with the exception of Case 2. The cholesterol findings were normal except in Case 9, this patient also having diabetes.

These results agree with the findings of Fahraeus,[16] Kovacs,[21] and Rubin,[22] in cases of carcinoma with or without jaundice.

2. *Catarrhal Jaundice* (Table II).—Catarrhal jaundice is not a disease entity. It consists, possibly, of two groups, one of which has a predominating infectious element (cholangitis), which may be designated as infectious hepatitis. The sedimentation rate is rapid in such cases. The other group begins insidiously without any infectious element. The term hepatosis, sug-

gested by Geronne,[23] is more appropriate for this particular condition, indicating a parenchymatous change of the liver cells, which was found by Eppinger,[24] Klemperer,[25] and others. In such cases leucin and tyrosin are occasionally present in the urine. Hepatosis not only occurs in young adults, but may also appear in later life, at which time it is difficult to differentiate from carcinoma with involvement of the liver bile ducts. The disease is not usually fatal; in a few cases, however, the lesions are so extensive that acute or subacute yellow atrophy of the liver may supervene. The etiology of the condition is still obscure. Sixteen cases of our series showed marked variations in the sedimentation rate. The sedimentation time was rapid in seven cases and normal or somewhat delayed in nine others. Apparently the presence or absence of bile in the stools does not affect the sedimentation rate. The cholesterol content of the blood was normal except in Case 1. This particular patient showed a hypercholesterolemia, secondary anemia, and diabetes mellitus. The jaundice subsided later.

TABLE II

HEPATOSIS (CATARRHAL JAUNDICE)

CASE	S.R.	L.F.T. %	I.I.	CHOL.	COMMON DUCT OBSTRUCTION	REMARKS
1. M.A.	18	100	135	412 mg.	Complete	Secondary anemia, amyloidosis
	10	100	120	570 mg.	Complete	Two weeks later
	85	15	25		None	Two months later
2. M.H.	30	10	45		None	
3. E.M.	43	45	45	140 mg.	Complete	
4. P.T.	45	90	160	170 mg.	Complete	
	70		32		None	Three weeks later
5. A.S.	60	15	24	148 mg.	None	
6. B.H.	65	45	35	164 mg.	None	
7. F.S.	90	80	100	208 mg.	Complete	
8. A.S.	110	35	30	230 mg.	Complete	
9. N.R.	120	70	72		Complete	
10. M.C.	200	50	55	208 mg.	Complete	
11. M.C.	210	100	60	154 mg.	Complete	
12. A.W.	300	100	122		None	
	215	40	80	260 mg.	None	Nine days later
13. L.S.	330	60	40		Complete	
14. C.S.	370	70	60	142 mg.	Complete	Pregnancy
15. W.C.	450	70	50	176 mg.	None	
16. A.A.	465	45	110	181 mg.	None	

Gram[12] reported a normal sedimentation time and normal blood fibrin in a case of catarrhal jaundice. Vorschütz[26] found an increased sedimentation rate in three cases of catarrhal jaundice (one to two hours). Kovacs[21] noticed a normal or slow sedimentation rate in four cases of catarrhal jaundice. He suggested the sedimentation time as a valuable diagnostic aid, as catarrhal jaundice shows a normal sedimentation rate and carcinoma with obstruction of the common duct shows a rapid rate. According to our results we cannot consider a rapid sedimentation rate as sufficient to exclude catarrhal jaundice but believe that a normal or slow sedimentation rate is suggestive of this condition.

3. *Cirrhosis of the Liver* (Table III).—In cirrhosis of the liver the sedimentation rate shows marked variations. In nine of our cases there was in-

creased sedimentation of the red cells and in three the rate was normal. The importance of the clinical course and other blood findings in these cases must not be disregarded, nor should too great reliance be placed on the sedimentation rate which may be modified by the possible presence of anemia. The bromsulphthalein liver function test offers considerable information concerning the condition of the liver, especially with a low icterus index. The high cholesterol content of the blood, in Case 1, was associated with amyloidosis and tuberculosis of the lymph nodes. An exploratory operation on this patient showed a moderate cirrhosis of the liver. The cases of splenomegalic cirrhosis of the liver and Banti's disease had the characteristic blood picture of anemia, leucopenia and thrombocytopenia. The sedimentation time was more rapid in cases with a severe anemia. Other cases of cirrhosis of the liver without jaundice were not included in this group, but their sedimentation time was observed to be normal.

TABLE III

CIRRHOSIS OF LIVER

CASE	S.R.	L.F.T. %	I.I.	CHOL.	COMMON DUCT OBSTRUCTION	REMARKS
1. M.S.	11	100	50	568 mg.	None	Tuberculosis of lymph nodes
2. T.M.	11	30	27	169 mg.	None	Splenomegalic cirrhosis
3. H.S.	15	65	75	356 mg.	None	Severe anemia Banti's Disease
4. R.C.	17	8	14	164 mg.	None	Splenomegalic cirrhosis
5. A.S.	24	30	35	356 mg.	None	Atrophic cirrhosis anemia
6. M.K.	48	10	12	136 mg.	None	Atrophic cirrhosis ascites
7. T.B.	62	90	77	240 mg.	None	Atrophic cirrhosis of the liver
8. F.W.	70	100	180	260 mg.	None	Atrophic cirrhosis
9. A.P.	72	20	7	142 mg.	None	Ascites, severe anemia
10. S.P.	180	20	9	136 mg.	None	Atrophic cirrhosis ascites
11. R.M.	182	60	30	176 mg.	None	Atrophic cirrhosis ascites
12. A.B.	300	35	13		None	Banti's Disease

4. *Lues* (Table IV).—Determinations of the sedimentation rate in cases of luetic hepatitis in the secondary stage were not made in our series, but, according to the observations of Klopstock,[27] these cases show a rapid sedimentation time. The cases of lues under our observation gave a definite history of long-continued salvarsan treatment prior to the onset of the jaundice. We found the sedimentation time to be rapid in four out of six subjects. In one the sedimentation time was markedly increased on account of an unusual com-

TABLE IV

LUES

CASE	S.R.	L.F.T. %	I.I.	CHOL.	COMMON DUCT OBSTRUCTION	REMARKS
1. D.A.	13	65	90		None	Schistosomiasis of liver. Post salvarsan
2. A.K.	54	70	55	142 mg.	Complete	Post salvarsan
3. N.H.	75	70	105		Complete	Post salvarsan
	55	90	182		Complete	One month later
	39	5	20		None	Six weeks later
4. S.F.	70	50	40	240 mg.	None	Post salvarsan
5. H.S.	140	90	80	208 mg.	None	Post salvarsan
6. A.M.	540	90	105	126 mg.	Complete	Post salvarsan

plication of schistosomiasis of the liver. Klopstock reported a normal sedimentation rate in two cases of hepatitis following salvarsan administration.

5. *Miscellaneous Cases* (Table V).—The sedimentation rate was found to be increased in all cases complicated by infection or anemia. In Case 12 a case of polycythemia vera complicated by jaundice and anemia as a result of the use of phenylhydrazine, the sedimentation rate increased. Previously, the sedimentation rate in this patient was extremely slow, the red blood cells having sedimented about 5 mm. after two days. Case 16, a man of sixty-two, showed marked jaundice which at first appeared to be due to carcinoma. Leucin and tyrosin were found in the urine, and it was suspected that marked

TABLE V

MISCELLANEOUS CASES

CASE	S.R.	L.F.T. %	I.I.	CHOL.	COMMON DUCT OBSTRUCTION	DIAGNOSIS
1. E.H.	11	20	36	130 mg.	None	Pregnancy, toxic hepatitis. Chills and fever
2. N.K.	14	35	45	320 mg.	Partial	Stricture of common duct
3. M.L.	15	0	18	190 mg.	None	Anemia, thrombosis of portal vein
4. I.S.	16	15	12	320 mg.	None	Cholecystitis
5. H.M.	20	50	55		None	Lymphatic leukemia
6. D.S.	23	10	21	156 mg.	None	Cholangitis. Chills and fever. Anemia
7. A.A.	25	65	85	190 mg.	Incomplete	Cholangitis
8. B.P.	28	85	45	260 mg.	Complete	Common duct stone
9. A.E.	42	0	24	160 mg.	None	Acute rheumatic fever
10. S.S.	45	15	35	280 mg.	None	Cholelithiasis
11. C.S.	65	45	52	80 mg.	None	Cardiac decompensation
12. B.R.	70	5	25		None	Polycythemia, jaundice following phenylhydrazine
13. H.T.	75	85	90		Complete	Common duct stone
14. H.L.	95	40	20	260 mg.	None	Leukemia
15. C.O.	105	15	18		None	Cardiac decompensation
16. J.W.	110	70	100	160 mg.	None	Subacute yellow atrophy
	38	35	70			2 weeks later
17. E.D.	120	70	72	230 mg.	Partial	Stricture of common duct

liver degeneration was present. Although his general condition became worse, the icterus index and liver function test showed some improvement. This indicated that we were not dealing with a carcinoma. The sedimentation rate also became more rapid (110 minutes to 38 minutes). The patient died, and at autopsy a subacute yellow atrophy of the liver was found. In two cases of common duct obstruction due to stone the sedimentation rate was found to be rapid.

SUMMARY

As shown by the following résumé of our cases, there is but slight variation in the sedimentation time of the various groups in which jaundice was present:

1. Catarrhal jaundice (16 cases). Sedimentation time 18 minutes to 465 minutes.

2. Carcinoma and jaundice (18 cases). Sedimentation time 11 minutes to 360 minutes.

3. Cirrhosis of the liver (12 cases). Sedimentation time 11 minutes to 300 minutes.

4. Lues and jaundice (6 cases). Sedimentation time varies from 13 minutes to 540 minutes.

5. Miscellaneous (17 cases). Sedimentation time 11 minutes to 120 minutes.

It therefore seems evident that the sedimentation reaction is only of value in certain cases of jaundice provided other data are available, such as the degree of liver impairment as determined by the bromsulphthalein test, intensity of the jaundice, and the presence of common duct obstruction or bile retention (as in hepatosis). The presence of bile in the urine and observation of the color of the stools are important factors in determining the existence of complete or partial obstruction of the bile ducts. The symptoms of the patient and the clinical course are considerations which must greatly influence the diagnosis.

The general variations of the sedimentation time are about the same in all varieties of jaundice. Comparable variations of the icterus index, cholesterol, and the results of liver function tests with bromsulphthalein can be noted in the various groups (see tables). The sedimentation time in catarrhal jaundice tends to be slow and in carcinoma with jaundice, it tends to be rapid in the majority of cases. This is an important differential diagnostic point in cases of afebrile jaundice when either condition is suspected.

The underlying factors which influence the variations in the sedimentation time have not been determined. The experiments in vitro with bile salts do not correspond with the sedimentation rate of red cells in icteric plasma. Even the relation of fibrinogen and other proteins to the sedimentation reaction of icteric blood is not definite, as certain cases of acute and subacute yellow atrophy of the liver which is accompanied by a marked diminution of the fibrinogen, have shown a rapid sedimentation time of the red cells. The factors which modify the sedimentation rate in icteric plasma require further study.

CONCLUSIONS

1. The sedimentation time of the blood is not a specific diagnostic or prognostic procedure in diseases accompanied by jaundice.

2. The majority of cases with jaundice show an increased sedimentation rate of the red blood cells.

3. Subnormal or normal variations of the blood cholesterol apparently have no effect on the sedimentation rate, but hypercholesterinemia (over 300 mg.) is accompanied by an increased sedimentation rate.

4. The sedimentation time in most of the cases of carcinoma and cholelithiasis with or without obstruction of the common duct is rapid. A normal sedimentation time in a jaundiced patient (without polycythemia) is suggestive of hepatosis (catarrhal jaundice). In other conditions of the blood and liver accompanied by jaundice, the sedimentation time shows marked variations. These depend upon infection, anemia and other unknown factors.

REFERENCES

1. Rosenthal, S. M., and White, E. C.: Clinical Application of the Bromsulphthalein Test for Hepatic Function, J. A. M. A. **84**: 1112-1114, 1925.
2. Katz, G., and Leffkowitz, M.: Die Blutkörperchensenkung, Ergebn. d. inn. Med. u. Kinderh. **33**: 266-392, 1928.
3. Linzenmeier, G.: Kritisches Sammelreferat über die Blutkörperchensenkungsgeschwindigkeit, Deutsche med. Wchnschr. **48**: 1023-1024, 1922.
4. Brown, A. L.: Rapid Clinical Method for the Determination of the Icterus Index, Arch. Path. **3**: 409-410, 1927.
5. Meulengracht, E.: The Clinical Importance of the Search for Bile Pigment in the Serum, Ugesk. f. Laeger. **81**: 1785-1799, 1919.
6. Shattuck, H. F., Killian, J. A., and Preston, M. A.: Comparison of Quantitative Methods for the Bilirubin of the Blood, J. Lab. & Clin. Med. **12**: 802-810, 1927.
7. Vorschütz, Johannes, and Joseph: Die Bedeutung der Hämogglutination und Bakterienagglutination als Diagnostikum und ihre Erklärung, Mitt. a. d. Grenzgeb. d. Med. u. Chir. **34**: 662-677, 1922.
8. Katz, G., and Radt, P.: Die Blutkörperchensenkung beim Ikterus, Med. Klin. **23**: 760-761, 1927.
9. Kürten, H.: Die Senkungsgeschwindigkeit der roten Blutkörperchen in ihrer Beziehung zu Cholesterin und Lecithin, Pflüg. Arch. f. d. ges. Physiol. **185**: 248-261, 1920.
10. Starlinger, W.: Ueber das Verhalten der zirkulierender Eiweisskörper des menschlichen Plasmas unter normalen und krankhaften Bedingungen. (Untersuchungen über Verteilungsverhältnis und physikochemische Konstanten) Deutsche med. Wchnschr. **54**: 731-733, 1928.
11. Whipple, G. H.: Fibrinogen. I. An Investigation Concerning Its Origin and Destruction, Am. J. Physiol. **33**: 50-69, 1914.
12. Gram, H. C.: The Results of a New Method for Determining the Fibrin-Percentage in Blood and Plasma, Acta Med. Scandinav. **56**: 107-161, 1922.
13. Rosenthal, F., and Wislicki, L.: Gallensäuren-Studien am ikterischen Menschen, Klin. Wchnschr. **6**: 781-784, 1927.
14. Shattuck, H. F., Katayama, I., and Killian, J. A.: Bile Pigments and Bile Acids of the Blood in Jaundice, Am. J. M. Sc. **175**: 103-121, 1928.
15. Byrom, F. B., and Kay, H. D.: Blood Phosphorus in Health and Disease. III, A. The Alleged Value of Blood Phosphorus Determinations in Suspected Malignant Disease. B. The Phosphorus Distribution in Anaemia, Polycythaemia and Leukaemia, Brit. J. Exper. Path. **9**: 72-81, 1928.
16. Fahraeus, R.: The Suspension-Stability of the Blood, Acta. Med. Scandinav. **55**: 1-228, 1921.
17. Pinner, M., Knowlton, K., and Kelly, R. G.: Sedimentation Rate of Erythrocytes; Its Relation to Fibrin Value and Cholesterol Content and Its Application in Tuberculosis, Arch. Path. **5**: 810-827, 1928.
18. Gram, H. C.: On the Causes of the Variations in the Sedimentation of the Corpuscles and the Formation of the Crusta Phlogistica ("size," buffy coat) on the blood, Arch. Int. Med. **28**: 312-330, 1921.
19. Frisch, A., and Starlinger, W.: Ueber die klinische Verwertung der Senkungsgeschwindigkeit der Erythrocyten bei der Lungentuberkulose, Med. Klinik. **17**: 1147, 1921.
20. Löhr, W.: Die Senkungsgeschwindigkeit der roten Blutkoerperchen als diagnostisches Hilfsmettel bei chirurgischen Erkrangungen Mitt. a. d. Grenzgeb. d. Med. u. Chir. **34**: 229, 1927.
21. Kóvacs, K.: Die Wert der Senkungsreaktion der roten Blukörperchen bei inneren Erkrankungen, Deutsche med. Wchnschr. **49**: 785-786, 1923.
22. Rubin, E. H.: Sedimentation Reaction in Cancer, Am. J. M. Sc. **174**: 680-690, 1927.
23. Geronne, A.: Zur Pathogenese einiger Formen des Ikterus, Klin. Wchnschr. **1**: 828-832, 1922.
24. Eppinger, H.: In Kraus and Brugsch: Spezielle Pathologie und Therapie innerer Krankheiten. Berlin. Urban and Schwarzenberg. Vol. 6, pt 2, p. 200, 1923.
25. Klemperer, P., Killian, J. A., and Heyd, C. G.: Pathology of "Icterus Catarrhalis," Arch. Path. **2**: 631-652, 1926.
26. Vorschütz, J.: Verschiedene Hämagglutinationsbilder bei Ikterusfaellen und ihre Bedeutung, Arch. f. Exper. Path. u. Pharmakol. **95**: 235-237, 1922.
27. Klopstock, E.: Catarrhal and Syphilitic Jaundice, Med. Klin. **19**: 1156-1157, 1923.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LABORATORY TECHNIC

IODINE CONTENT OF FOODS: Estimation of Iodine in Foodstuffs and Body Fluids, Leitch, I., and Henderson, J. M. Biochem. J. London. 22: 1003, 1926.

Reagents and apparatus required:

1. Potassium hydroxide sticks: iodine-free.
2. Alcohol for extraction: 95 c.c. absolute alcohol, 5 c.c. distilled water.
3. Methyl orange: 0.05 per cent *watery* solution.
4. Sulphuric acid: approximately 2 N.
5. Bromine water: to be prepared as required.
6. Sodium thiosulphate: approximately N/500. This solution should be made up periodically from N/10 stock solution. N/500 thiosulphate does not keep well and must be standardized daily by means of potassium bi-iodate as described by Kendall (1920).
7. Solution of potassium iodide made up just before the final titration. A small crystal is dissolved in about 20 c.c. of water.
8. Starch solution: to 50 c.c. distilled water add a pinch of soluble starch. Boil for one to two minutes and cool. A fresh solution should be prepared every third day.
9. Small chips of pumice stone, about the size of rice grains. These chips, after removing dust through a fine sieve, are boiled in dilute nitric acid, washed with distilled water, dried, and then strongly heated in the crucible furnace. After the estimation they are recovered. From time to time they are retreated and used over again.
10. Davis combined crucible furnaces with Teelu burners. Vitreosil basins for the sand are most suitable.
11. Nickel crucibles (6 cm. and 5 cm. in diameter). Nickel stirring rods.
12. Flasks (50 c.c. capacity).
13. A serum pipette, 0.1 c.c. capacity graduated in thousandths of a c.c.

PROCEDURE

Ashing.—A suitable quantity of the substance to be analyzed is measured into a nickel crucible (6 cm. diam.) and 1 gm. of potassium hydroxide stick added, dissolved in water in the case of dry substances. The contents of the crucible are stirred and then heated gently over a Bunsen burner until all bubbling ceases, and thereafter more strongly until no more fumes come off. Heating is continued on the furnace to a dark gray ash. The crucible must not be allowed to glow. When cool the ash is moistened with distilled water and carefully heated over a low flame until dry. The crucible is then returned to the crucible furnace and heated until no further change appears to be taking place (say ten minutes). After cooling, about 5 c.c. of distilled water are added and the contents of the crucible filtered through a No. 40 Whatman ash-free filter-paper. (In all pouring operations it is necessary to vaselin the lip of the crucible with iodine-free vaselin.) The filter-paper is washed three times with about 5 c.c. of water. The filtrate, collected in a small beaker, is set aside. It should be clear, or at most, only very faintly yellow.

The filter-paper with its charred contents is returned to the nickel crucible, dried and then heated in the crucible furnace until a clean ash is obtained. The crucible is cooled, the filtrate returned to it and evaporated to dryness. This operation requires the greatest caution towards its completion, since spurting and crackling may cause loss. The crucible is again heated in the furnace for a minute or so, then cooled and about 3 c.c. of water are added. This thick solution is gently evaporated over a low Bunsen flame until a skin begins to form on the surface. Then the contents, after cooling, are ready for extraction.

Extraction.—The ash is extracted three times with 3 c.c. of the alcohol solution, the extract being filtered through a No. 40 Whatman paper into a 5 cm. crucible. The ash

should form a smooth paste. If it is gritty one or two drops of water should be added, but excess should be avoided. After the third extraction the paste is poured on to the filter and the last of the alcohol allowed to drain. The filter-paper is rejected, the funnel washed with a little distilled water and the filtrate evaporated to dryness on the water-bath.

The thin film of salt on the bottom of the crucible should be practically free from carbon. To get rid of any small particles which may be present, the bottom of the crucible is glowed gently for a few seconds, the salt film being just allowed to melt.

When the crucible has cooled the contents are dissolved in distilled water, and transferred to a 50 c.c. flask.

Titration.—Two drops of the methyl orange solution are added. The solution is rendered neutral by the addition, drop by drop, of 2N sulphuric acid. One drop is added after the neutral point. In all about three drops are generally required. Thereafter a few drops of bromine water are added. The red color is immediately discharged. About 1 c.c. of bromine water should be added in excess of this, until the solution is distinctly yellow.

Three or four pieces of pumice stone are added and the solution is boiled over a medium flame, until the volume is reduced to between 1 and 2 c.c., and then cooled. Two drops of the potassium iodide solution and two drops of the starch indicator are added and the titration carried out with N/500 thiosulphate from the 0.1 c.c. pipette. By holding the flask at an angle it is possible by tapping the point of the pipette to run in as little as 0.001 c.c. at a time. If due precautions have been taken, the end-point is perfectly definite and after a little practice may be determined to about 0.002 c.c.

Calculation:

$$\frac{\text{Titration figure x iodine equivalent of thiosulphate}}{9}$$

gives the amount of iodine in the sample.

PROTEIN DETERMINATION: Colorimetric Determination of Proteins in Plasma, Cerebrospinal Fluid, and Urine, Wu, H., and Ling, S. Chinese J. Physiol. **1**: No. 2, 161, 1927.

Standard solution: Tyrosine 50 gm. dissolved in 250 c.c. N/0.1 HCl. Phenol Reagent:

$Na_2WO_4 2H_2O$	100 gm.
$Na_2MoO_4 2H_2O$	25 gm.
Water	700 c.c.

Dissolve and add 50 c.c. of 85 per cent H_3PO_4 and 100 c.c. concentrated HCl. Boil with condenser eight hours and dilute to one liter.

DETERMINATION OF PROTEINS IN PLASMA

Determination of Fibrin: To 1 c.c. of the plasma, from blood containing 0.2 to 0.6 per cent potassium oxalate, add 28 c.c. of 0.8 per cent NaCl solution and 1 c.c. of 2.5 per cent $CaCl_2$ solution. Mix and allow to stand undisturbed until a solid clot has formed. Break up the jelly by shaking slightly and transfer it to a dry filter. While filtering insert into the jelly a slender glass rod with a pointed end and whirl gently. All the fibrin will stick to the rod. Slip the fibrin off the rod, and press it between dry filter-paper to remove the adhering liquid. Transfer it to a 15 c.c. centrifuge tube, add 4 c.c. of 1 per cent sodium hydroxide. Place the tube in a boiling water-bath and stir with a slender glass rod until the fibrin lump has completely disintegrated leaving the calcium oxalate in suspension. Add 10 c.c. of water, mix, and centrifuge. Transfer the supernatant liquid to a 25 c.c. volumetric flask or graduated tube. Cool under the tap. Add 1 c.c. of 5 per cent H_2SO_4, 0.5 c.c. of phenol reagent, and dilute to about 20 c.c. Add 1 c.c. of 10 per cent NaOH solution. Mix by gentle rotation, make up to volume.

The standard is prepared as follows: Measure 1 c.c. of the standard tyrosine solution into a 25 c.c. volumetric flask or graduated tube, add 0.5 c.c. of phenol reagent, dilute to about 20 c.c. and finally add 1 c.c. of 10 per cent NaOH solution. Make up to volume and mix. The standard should, of course, be prepared at the same time as the unknown. Let stand for one hour before making the color comparison.

If any fibrin fails to stick to the rod it should be picked up with the tip of the rod. If the amount of the fibrin in the plasma is very high, say 0.8 per cent or more, the fibrin jelly will not shrink readily. In such a case it is necessary to use less of the plasma for the determination.

Calculation: If the standard is set at 20 and the reading of the unknown is R, then the amount of the apparent tyrosine determined is $\frac{20}{R} \times 0.2$ mg. Since 1 mg. of tyrosine = 11.3 mg. of fibrin, the amount of fibrin in 1 c.c. of plasma is $\frac{20}{R} \times 0.2 \times 11.3$ mg. or the percentage of fibrin $= \frac{20}{R} \times 0.226$.

Determination of Albumin: To 1 c.c. of plasma add 4 c.c. of H_2O with 5 c.c. of saturated ammonium sulphate solution; or 9 c.c. of saturated magnesium sulphate solution and 0.3 gm. of anhydrous magnesium sulphate. Mix and allow to stand for thirty minutes. Filter. Measure 1 c.c. of the filtrate into a 15 c.c. centrifuge tube. Add about 12 c.c. of H_2O, 1 c.c. of 10 per cent sodium tungstate solution, and then 1 c.c. of 2/3 N sulphuric acid. It is advisable to add one or two more drops of acid. Stir thoroughly with a slender glass rod and centrifuge. Carefully decant the supernatant liquid as completely as possible. The volume of the wet precipitate usually amounts to about 0.5 c.c. If it is much smaller than 0.5 c.c. indicating low albumin, measure another c.c. of albumin filtrate into the same tube, dilute with water and proceed as before. Add to the precipitate in the centrifuge tube 1 c.c. of sodium tungstate solution. Stir until the precipitate has dissolved, dilute with 13 c.c. of H_2O, and add 1 c.c. of H_2SO_4. Stir again, centrifuge, and decant the supernatant fluid. This second precipitation is intended to remove the calcium and ammonium or magnesium so nearly completely that they cannot possibly interfere with the subsequent color reaction, although experience has shown that a single precipitation usually suffices. Add to the precipitate in the tube 10 c.c. of H_2O and 1 or 2 drops (but no more) of 20 per cent Na_2CO_3. Stir until the precipitate has dissolved. Transfer the resulting solution to a 25 c.c. volumetric flask or graduated tube. Rinse the centrifuge tube twice with 3 c.c. of H_2O. Add 0.5 c.c. of phenol reagent and 1 c.c. of 10 per cent NaOH solution. Mix by gentle rotation. Make up to volume and mix. Prepare a standard as in the fibrin determination and read the color after one hour.

Determination of Albumin and Globulin: Measure 2 c.c. of the filtrate in the fibrin determination into a 15 c.c. centrifuge tube and proceed exactly as in the determination of albumin.

Calculation: In the calculation it is to be noted that the solution used for the albumin determination is plasma diluted 1 to 10, while that used for the determination of albumin and globulin is plasma diluted 1 to 30. If 1 c.c. of the albumin solution is used for the former determination and 2 c.c. of the serum solution are used for the latter determination, and the colorimeter readings are R_a and R_t respectively, the standard being set at 20, then the total apparent tyrosine in 1 c.c. of serum $= 15 \times \frac{20}{R_t} \times 0.2$ mg. the apparent tyrosine of albumin in 1 c.c. of serum $= 10 \times \frac{20}{R_a} \times 0.2$ mg. and the apparent tyrosine of globulin in 1 c.c. of serum is

$$(15 \times \frac{20}{R_t} \times 0.2) - (10 \times \frac{20}{R_a} \times 0.2) = \frac{60}{R_t} - \frac{40}{R_a} \text{ mg.}$$

Since 1 mg. of tyrosine = 15.8 mg. of globulin = 21.4 mg. of albumin of human serum

$$\text{per cent of globulin} = \frac{(\frac{60}{R_t} - \frac{40}{R_a}) \times 15.8}{1{,}000} \times 100 = (\frac{6}{R_t} - \frac{4}{R_a}) \times 15.8$$

$$\text{per cent of albumin} = \frac{(10 \times \frac{20}{R_a} \times 0.2) \times 21.4}{1{,}000} \times 100 = \frac{20}{R_a} \times 4.28.$$

DETERMINATION OF PROTEIN IN URINE

Transfer to a 15 c.c. centrifuge tube 1.5 c.c. of filtered urine and dilute with water to about 10 c.c. Add 2 c.c. of 10 per cent sodium tungstate and 2 c.c. of 2/3 N sulphuric acid. The mixture is stirred thoroughly with a slender glass rod and centrifuged. In case the supernatant liquid is milky in appearance which occurs only in alkaline urine, add a few more drops of 2/3 N sulphuric acid and the mixture is stirred and centrifuged again. The water clear supernatant liquid is carefully decanted. To the precipitate in the centrifuge tube add 1 c.c. of sodium tungstate and stir until it has completely dissolved. Dilute with about 10 c.c. of water and add 1 c.c. of 2/3 N sulphuric acid. The mixture is again stirred, centrifuged, and the supernatant liquid decanted. This procedure is repeated once more in order to remove completely those nonprotein substances which react also with the phenol reagent. Finally, the precipitate is suspended in 10 c.c. of water and dissolved with one or two drops of 20 per cent sodium carbonate solution. If the volume of the wet precipitate amounts to about 0.4 c.c. the whole solution is transferred to a 25 c.c. volumetric flask or graduated tube and the centrifuge tube rinsed twice with 3 c.c. of water. If the volume of the precipitate is much smaller than 0.4 c.c. the determination should be repeated with more urine. If it is more than 0.5 c.c. the final solution is made up to 10 c.c. and an aliquot portion is used for the determination. The rest of the procedure is the same as described above.

Calculation: If the standard is set at 20 and the reading of unknown is R, then the apparent tyrosine determined is $\frac{20}{R} \times 0.2$ mg.

Since the protein in the urine is largely albumin, 1 mg. tyrosine may be taken as equivalent to 20 mg. urinary protein. If the amount of urine used is n c.c. the amount of protein is 100 c.c. urine is $\frac{20}{R} \times 0.2 \times 20 \times \frac{100}{n}$ mg. $= \frac{20}{R} \times \frac{400}{n}$ mg. or the percentage of protein in urine is $\frac{20}{R} \times \frac{4}{10n}$.

DETERMINATION OF PROTEIN IN CEREBROSPINAL FLUID

Transfer to 15 c.c. centrifuge tube 2 c.c. of spinal fluid. Add 3 c.c. H_2O followed by 1 c.c. each of 10 per cent sodium tungstate and 2/3 N sulphuric acid. The mixture is stirred and centrifuged. Decant the supernatant liquid. Add to the precipitate 1 drop of 10 per cent Na_2CO_4 and H_2O to the 5 c.c. mark. Stir until the precipitate has dissolved. Add 0.2 c.c. of phenol reagent and 1 c.c. of 5 per cent NaOH. The content of the tube is mixed by inversion. After one hour the color is compared in a usual manner against a standard prepared at the same time as the unknown.

Two standards are prepared as follows: Into two similar test tubes marked Standard 1 and Standard 2 are measured 0.2 c.c. and 0.4 c.c. of the standard tyrosine solution, and 4.8 c.c. and 4.6 c.c. H_2O respectively. Then add to each 0.2 c.c. of phenol reagent and 1 c.c. of 5 per cent sodium hydroxide. The solutions are mixed by inverting the tubes. The standard which is closer, by inspection, to the unknown is used for the comparison.

Calculation: We have not been able to collect enough cerebrospinal fluid for the determination of the tyrosine equivalent by the Kjeldahl method. For clinical purposes the tyrosine equivalent of the protein in spinal fluid may be taken as 18, the average of the value for serum albumin and globulin. If Standard 1 is used and set at 15, then $\frac{15}{R} \times 0.04 \times \frac{100}{2} \times 18 =$ mg. of protein per 100 c.c. of spinal fluid. With Standard 2 set at 15, $\frac{15}{R} \times 0.08 \times \frac{100}{2} \times 18 =$ mg. of protein per 100 c.c. of spinal fluid.

DETERMINATION OF SOLUBLE PROTEINS

The colorimetric method is especially useful for comparing relative concentrations of protein solutions. If the protein solutions contain no interfering substances, suitable volumes are measured into 25 c.c. graduated tubes and the color developed directly. If the solutions

contain interfering substances, the protein is first precipitated with suitable amounts of sodium tungstate and sulphuric acid.

If it is desired to calculate the absolute concentration of the protein, it is of course necessary to determine beforehand the tyrosine equivalent of the protein in question by paralled tyrosine and nitrogen determinations.

CEREBROSPINAL FLUID: The Fuchsin-Sublimate Reaction (Takata-Ara), Kafka. München. med. Wchnschr. 73: 1836, 1926.

The practical execution of Takata and Ara's new cerebrospinal fluid reaction consists in the mixing and shaking thoroughly of 1 c.c. of cerebrospinal fluid with a drop (0.033 c.c.) of a 1 per cent sodium carbonate solution and 0.3 c.c. of a freshly prepared mixture of equal parts of a 0.5 per cent sublimate and a 0.2 per cent fuchsin solution. The normal cerebrospinal fluid shows a bluish-purple color and remains clear.

In case of a syphilitic nervous system, which has already changed the protein fraction of the cerebrospinal fluid, a flocculation with bluish-purple sediment appears, above which is a transparent liquid.

In case of a nonsyphilitic meningitis with a protein quotient similar to the protein content of the serum a rose discoloration without deposit, is observed.

Four degrees of flocculation can be distinguished in the syphilis type of the reaction, the intensity of which is dependent upon the length of the time until the sediment appears. An immediate total flocculation indicates the highest degree while 3 slighter ones are observed after fifteen and thirty minutes and twenty-four hours.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Practical Physiological Chemistry**

IN REVIEWING the eighth edition of Hawk and Bergeim's *Practical Physiological Chemistry*, we remarked that a volume which has passed through as many editions requires no introduction. The ninth edition, however, increased to nearly double the size and containing a vast amount of new and valuable information may almost be looked upon as a new creation. Many of the chapters have been written by collaborators especially qualified by their particular lines of study. We would mention particularly the chapters on respiratory metabolism and neutrality regulation, energy metabolism, and studies in metabolism.

Hawk and Bergeim's *Practical Physiological Chemistry* will continue to hold the high recognition which it has earned for itself.

How to Make the Periodic Health Examination†

ANY innovation in the art of medicine is viewed with some apprehension by the uninitiated until on closer study it is found that in a great part at least we are dealing with the same old theory dressed up in new clothes. How to Make the Periodic Health Examination, might with equal justice have been entitled How To Make a Thorough Physical Examination. The one unusual feature and one which should be of greatest assistance to those who are not in the habit of making thorough physical examinations lies in the fact that the program outlined, if followed, will enable efficient systematization, will promote thoroughness, and will thereby help to prevent diagnostic oversight. Adherence to the routine outlined in this manual will enable the examiner to recognize the earlier deviations from normal and the predispositions, and will give him the opportunity of preventing where possible the more serious late developments.

The man who has not yet developed a satisfactory record system will find many helpful samples in this volume. There is also a convenient reference section covering directions to patients particularly with regard to hygiene, diet, exercise, and the like.

The Life Extension Institute with its periodic health examination has developed a large volume of interesting statistical information particularly regarding early pathologic changes and predispositions, all of which is incorporated in the volume.

---

*Practical Physiological Chemistry. A book designed for use in courses in practical physiological chemistry in schools of medicine and of science. By Phillip B. Hawk, M.S., Ph.D., President of the Food Research Laboratories, Inc., New York City, and Olaf Bergeim, M.S., Ph.D., Assistant Professor of Physiological Chemistry in the University of Illinois, College of Medicine, Chicago. Ninth Edition, Revised and Enlarged. Cloth. Illustrated. Pages 931. P. Blakiston's Son & Co., Philadelphia, 1926.

†How To Make the Periodic Health Examination. A Manual of Procedure. By Eugene Lyman Fisk, M.D., Medical Director, Life Extension Institute and J. Ramer Crawford, M.D., Assistant Medical Director, Life Extension Institute. Foreword by Major General Merritte W. Ireland, Surgeon General, United States Army. Cloth. Pages 393. The Macmillan Company, New York, 1927.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

*Practical Lectures**

THIS work covers such a wide variety of subjects that it does not lend itself easily to review. The Medical Society of the County of Kings, Brooklyn, N. Y., instituted what might be termed a postgraduate course in which authorities on a wide variety of the aspects of medicine and surgery were invited to deliver lectures to the Society, lectures which are primarily practical in that they give forth information which may be applied with advantage in the routine practice of medicine. The present volume is the second of a series being published by this Society. It should be of great value to the general practitioner who will find between the covers a quite comprehensive postgraduate medical instruction.

The Medicine Man†

THOSE who enjoy medical biographies, and every physician should, will look forward to reading Dr. E. T. Dudley's autobiography. Here is the story of a man who actually did work his own way from even his primary education, on and upward to that soul-satisfying point where he had received the highest honors in his branch of the profession, had retired as president of a great medical school and had received the recognition of the medical world for his scientific contributions.

Dr. Dudley came from sturdy New England stock, started to earn his own way at the tender age of thirteen as a clerk in an apothecary shop, worked his way through Dartmouth and through Yale, graduated from Long Island Medical College, procured for himself the best possible training in gynecology under the associates of J. Marion Sims and then settled in Chicago where he grew with the city and where Northwestern University Medical School grew with him.

Most of us do not settle down to enjoy medical biography until we have reached that point where we find sufficient leisure in our busy day for this type of relaxation. A medical man at any age will enjoy this autobiography but its greatest inspiration will be to the young man of little or no financial resources who is contemplating the field of medicine as a lifetime study. Here he will find all of the encouragement he will need, but unfortunately it is he who will probably be least likely to discover the book.

An autobiography to be really thrilling must make somewhat of a hero out of the principal character. The great risk, therefore, becomes the appearance of too great egotism. Edward Bok wrote his autobiography in the third person so as to avoid the large I but his contribution is far more egoistical than that of Dr. Dudley.

His record of his adventures is simply recorded and with a wealth of dry and subtle humor. His deductions from his experiences displays the mental workings of a true philosopher. His fund of anecdote is charming.

We suspect that the auburn tint of Dr. Dudley's hair has been quite a factor in his usually getting what he set out after. The red headed boy is usually meat for his companions' buffoonery and in self-protection he develops a self-reliance and unshakable determination. Some day we hope to read a scientific dissertation on the psychology of the red-topped youngster.

Dr. Dudley takes us through the early days of medical apprenticeship, the two year medical school in which the second year lectures repeat the first year, the days of septic surgery, of antiseptic surgery and finally of aseptic surgery. We notice with surprise and with some regret that he has practically nothing to say of the development of medical education in the great Chicago school with which he was associated.

There is quite a scattering of typographic errors which, however, in no way detracts from the pleasure of the book.

---

*Practical Lectures on the Specialties of Medicine and Surgery. Delivered under the Auspices of The Medical Society of the County of Kings, Brooklyn, New York. (Second Series, 1924-1926.) With one hundred and ten illustrations. Cloth. Pages 590. Paul B. Hoeber, Inc. New York, 1927.

†The Medicine Man. Being the Memoirs of Fifty Years of Medical Progress. By E. C. Dudley, M.D., LL.D., Ex-President American Gynecological Society, President Emeritus of Northwestern University Medical School. J. H. Sears & Co. Inc., New York, 1927.

*Sulphur Metabolism**

THE late Dr. Kahn and Dr. Goodridge, while studying the biochemistry of sulphur compounds found that the task of covering the literature of sulphur compounds in plant and animal metabolism was enormous. They therefore decided that to aid others who might sometimes carry studies along similar lines it would be well to prepare an exhaustive résumé of the entire literature on the subject. This they have done in a volume of 831 pages.

They have not been content with merely recording the bibliography but have reported in detail those observations in the literature which to them appeared of primary importance together with a wealth of tabulation and have classified the literature and arranged it in coordinated sequence.

The great value of the work will, as the authors have indicated, be as a reference organ for the student of the metabolism of sulphur.

The Elements of General Zoology†

IN THE profusion of textbooks here is one that is truly different. The difference consists in that while most textbooks on elementary zoology stress the study of structure Dr. Dakin's text correlates the structural studies with functional studies. Much more space is given to the latter than to the former. Indeed, we might designate this volume as a work on comparative anatomy, physiology, and embryology of those forms of life which are usually studied in the zoologic laboratory.

Textbook of Medicine‡

A SINGLE volume of 1500 pages designed primarily as a student text and as a quick reference volume. There are 130 contributors to the volume every one of whom is an outstanding authority. Indeed, one marvels in looking over the list of contributors that the editor has succeeded in collecting such a galaxy of names.

The discussion of the individual diseases is necessarily brief but is at the same time concise and pointed. We anticipate that this textbook will come into quite general use.

The Normal Chest§

THE anatomy, physiology, embryology, roentgen, and physical aspects of the normal chest. The undergraduate medical student usually studies anatomy one year, physiology the next and takes up clinical studies after he has forgotten much of the former. The volume under consideration correlates all of these studies of the thorax into one systematized review. There is no discussion of pathology. The volume should be of value not only to undergraduate students but to all who wish from time to time to review concisely their knowledge on the subject of the chest.

---

*Sulphur Metabolism. A Review of the Literature. By Max Kahn, M.A., M.D., Ph.D., Associate in Biological Chemistry, School of Medicine Columbia University; Visiting Physician in Diseases of Metabolism and Director of Laboratories, Beth Israel Hospital; Visiting Physician United-Israel-Zion Hospital and Frederic G. Goodridge, M.D., Ph.D., Associate in Biological Chemistry, School of Medicine, Columbia University. Cloth. Pages 831. Lea & Febiger, Philadelphia and New York, 1926.

†The Elements of General Zoology. A Guide to the Study of Animal Biology Correlating Function and Structure with Notes on Practical Exercises. By William J. Dakin. D.Sc., F.Z.S., Professor Zoology in the University of Liverpool. Cloth. Illustrated. Pages 496. Oxford University Press, American Branch, New York, 1927.

‡A Textbook of Medicine. By 130 American Authors. Edited by Russell L. Cecil, M.D., Assistant Professor of Clinical Medicine. Cornell University, Medical School, New York. Octavo of 1500 pages, illustrated. Cloth. W. B. Saunders Company, Philadelphia and London. 1927.

§The Normal Chest of the Adult and the Child. Including Applied Anatomy, Applied Physiology, X-Ray and Physical Findings. By J. A. Myers. Associate Professor of Preventive Medicine, Medical and Graduate Schools, University of Minnesota; Medical Director, Lymanhurst School for Tuberculous Children, Minneapolis, Minn., 143 Illustrations, 191 References. Cloth. Pp. 400. The Williams & Wilkins Company, Baltimore, Md., 1927.

We would mention particularly the discussion of the lymphatic supply of the thorax, the discussion of routine systematic examination, innervation of the chest, the developmental anatomy of the chest, and the acoustics of percussion and auscultation. The illustrations are remarkably clear and descriptive, adding greatly to the value of the work.

---

*Tonic Hardening of the Colon**

GASTROINTESTINAL function, or rather dysfunction is receiving more and more attention. Possibly the day is not far distant when such diagnoses as dyspepsia, nervous indigestion, gastric neurosis, and intestinal indigestion will not be needed to cover up ignorance.

Wilson's monograph upon a common colonic condition is an important stepping stone toward a better understanding of the nervous dyspeptic. It throws new light upon spastic constipation. The work is divided into three parts: symptomatology, clinical section, physiologic considerations.

The general practitioner and gastroenterologist alike, will spend an interesting, as well as profitable, hour in the perusal of this monograph.

Pharmaceutical Bacteriology†

THIS is a textbook on bacteriology intended for the use of pharmacists.

It is questionable whether the pharmacist should be prepared to conduct a clinical laboratory and equally questionable whether this book will enable him to do so.

Brain and Heart‡

THE title of this small work is regrettably misleading and gives no conception of the material between the covers. The work consists essentially of a series of philosophic deductions based in part upon Fano's extensive experimental studies in physiology. The first portion might be aptly termed a physiologist's conception of the nature of living matter. The second might well be termed personal ideas on the origin of life and the development of species. Fano is inclined to doubt the theory that life ever developed out of an azoic period. Rather he says life has always existed and inorganic matter has developed as a result of the activities of life. He accepts neither Darwinism nor the Lamarckian philosophy.

Chronic Rheumatic Diseases. Their Diagnosis and Treatment§

THERE is a tale of a man who rushed into a busy practitioner's office and who, impatient of delay, demanded a prescription for rheumatism. He was given the prescription and as he was hurrying down the street was called back by the physician who said, "If the medicine helps you, be sure to let me know for I have rheumatism too."

The present treatment of chronic arthritis, almost entirely palliative, is on the whole so unsatisfactory as to be a source of regretful embarrassment to the physician.

---

*Tonic Hardening of the Colon. By T. Stacy Wilson, M.D., B.Sc. (Edin.), F.R.C.P. (Lond.) Cloth. Pages 210. Oxford University Press, American Branch, New York, 1927.

†Pharmaceutical Bacteriology. By Albert Schneider, M.D., Professor of Pharmacognosy, University of Nebraska, Second Edition, Cloth, 97 illustrations, 441 pages. P. Blakiston's Son and Co., Philadelphia.

‡Brain and Heart. Lectures on Physiology. By Giulio Fano of the Royal University of Rome. Translated by Helen Ingleby with a foreword by Prof. E. H. Starling, C.M.G., M.D., D.Sc., F.R.S., University College, London. Cloth. Pages 142. Oxford University Press. American Branch, New York, 1926.

§Chronic Rheumatic Diseases. Their Diagnosis and Treatment. By F. G. Thomson, M.A. Cantab., M.D., F.R.C.P. Lon. Physician to the Royal United Hospital, Bath Consulting Physician to the Royal Mineral Water Hospital, Bath, Etc., and R. G. Gordon, M.D., D.Sc., M.R.C.P., Edin. Physician to The Royal Mineral Water Hospital, Bath, Physician to The Children's Orthopedic Hospital, Bath, Assistant Physician to The Royal United Hospital, Bath. Cloth. Pages 202. Oxford University Press, American Branch, New York, 1926.

Dr. Crowe on the other hand is most optimistic and claims with his methods to have achieved excellent results. In the present monograph he presents his methods and results with a promise to give in a succeeding contribution more detailed presentation of his experimental work and laboratory methods.

The author first recognizes various types of arthritis and stresses the importance of knowing in so far as possible with what type the physician is dealing. He recognizes the beneficial effect of supportive and palliative therapy as is customarily applied but in his specific work he attempts to build up the individual's general resistance to invading bacteria. His treatment is, briefly, vaccine therapy, but vaccine therapy very carefully controlled. The two microorganisms which in his experience are mainly responsible are a Staphylococcus albus designated by him micrococcus deformans and a streptococcus. These are usually natural inhabitants of the intestinal tract and may be present in infectious foci.

The author recommends the use of a stock mixed vaccine of these organisms isolated from the various foci but particularly from the intestinal tract but emphasizes that his excellent results have been due to his carefully controlled dosage and administration. He uses small doses rather than large ones. There is so much detail in the supervision of administration that one desiring to follow the author's methods must perforce carefully study the monograph.

---

*Pathological Physiology of Internal Diseases**

A SAD and regrettable consequence of passage from this life, among medical writers at least, is that their works which previously may even have been standard reference literature gradually find their way to the dusty shelves to be delved into only by the student of past history. This is especially regrettable in the case of Hewlett's Functional Pathology for it is a contribution of utmost value and we owe a debt of gratitude to his colleagues who, on the basis of Hewlett's revisionary notes and their own knowledge and experience have brought his functional pathology up to the minute and dedicated it as a memorial volume.

The classifications and arrangements are practically unchanged for indeed they could scarcely have been improved upon.

We believe we are safe in saying that Hewlett's Functional Pathology either in its present form or as it originally appeared as a volume of Monographic Medicine will be found in as many private medical libraries as very nearly any other one medical treatise.

Lobar Pneumonia†

A COMPREHENSIVE presentation of the evolution and course of lobar pneumonia as recognized and studied by the roentgenologist. The volume is rather small, concise and at the same time quite inclusive. In most phases of the discussion the bacteriologic findings receive little attention. In the discussion of hilus pneumonia, we would have liked especially to have known the bacteriologic findings.

The author establishes convincingly his case to the effect that x-ray examination is of greatest assistance in the early recognition of pneumonia and especially in the early discovery of complications. We are rather disappointed not to see some mention based on his wide roentgen experience of abortive types of pneumonia, the so-called three day pneumonia. He leaves the question uncertain as to its existence.

---

*Pathological Physiology of Internal Diseases. Functional Pathology. By Albion Walter Hewlett, M.D., B.S. Formerly Professor of Medicine, Stanford Medical School, Professor of Internal Medicine and Director of Clinical Laboratory, University of Michigan. Revised in Memoriam by his Colleagues. Under the Editorial Supervision of George DeForest Barnett. With one hundred and sixty-four illustrations in text. Cloth. 787 pages. D. Appleton & Company, New York.

†Lobar Pneumonia. A Roentgenological Study. (A Correlation of Roentgen-Ray Findings with Clinical and Pathological Manifestations). By L. R. Sante, M D., F.A.C.R., F.A.C P. Associate Professor Radiology, St. Louis University Medical School; Radiologist to the University Group of Hospitals (St. Mary's Hospital and Infirmary); Chief City Radiologist to St. Louis City Hospitals; Consultant Radiologist Koch Hospital for Tuberculosis, St. Louis Training School, etc. Illustrated. Cloth. Pp. 137. Paul B. Hoeber, Inc., New York, 1928.

*Bacteriology and Surgery of Chronic Arthritis and Rheumatism**

IN CROWE'S first volume which has been reviewed in these columns he promised a second in which the details of technic would be discussed in greater detail. We have before us this second volume which we suspect has taken on a slight different form from the author's original intention, the new form developing naturally with the evolution of his discussion. A large first section continues to deal with the etiology of arthritis. This is followed by a critical study of his own therapeutic results, a rather brief discussion of bacteriologic technic (we had anticipated rather more), and a classification of the streptococci which he has obtained from arthritic cases. A chapter on physical treatment is contributed by Herbert Frankling.

Crowe's mode of attack on arthritis is from the bacteriologic viewpoint and his results arrest attention. The streptococcus is quite generally conceded to be a factor in arthritis but Staphylococcus albus has not been as positively incriminated by other workers.

This volume like its predecessor should be in the hands of all who undertake a serious study of arthritis.

Elements of Physiology for Students of Medicine and Advanced Biology†

HERE is an interesting work. We prick up our ears when we read the preface and then turn to the text with some apprehension for we have often read in prefaces of new modes of attack only to be disappointed on finding the same old routine presentation in the text.

In the preface the authors announce that the subject is presented by the development of two keynote principles. The first is that living protoplasm is a system of molecules and ions, hence, understanding of its structure and function is to be sought by study of those physical, physicochemical and chemical laws which govern the interrelations of molecules and ions. The first section therefore contains an account of the nature and capacities of protoplasm as developed primarily from a study of individual cells and their environmental reactions. The second key-principle is that every protoplasmic cell is inherently a self-sustaining system and provided its environment is right it should continue to live and function almost indefinitely.

In other words after reading the preface we hope to find a consecutive development of the principles of human physiology, along pure biologic lines beginning with the physiology of the individual cells and their reactions to environmental alterations and their developmental changes consequent on functional specialization.

To our surprise and delight we find that these authors have done what they announced they would do. The result is a quite comprehensive treatise on human physiology which reads more like a story book than textbook and in which the plot is gradually and logically developed.

Bolles Lee's Microtomist's Vade-Mecum‡

THIS volume is so well known to the tissue pathologist that its ninth edition requires no introduction. It is sufficient to say that the present edition of Lee's monumental work is edited by Gatenby of Dublin and Cowdry of New York with the collaboration of eleven men preeminent in their field. We might describe the work as the standard reference encyclopedia for tissue pathologists.

---

*Bacteriology and Surgery of Chronic Arthritis and Rheumatism With End-Results of Treatment. By Warren Crowe, D.M., B.Ch. (Oxon.), M.R.C.S., L.R.C.P. The Chapter on Surgical Treatment by Herbert Frankling C.B.E., M.R.C.S., (Eng.) Cloth. Illustrated. Pp. 187. Oxford University Press, American Branch, New York. 1927.

†Elements of Physiology for Students of Medicine and Advanced Biology. By Earnest G. Martin, Ph.D. Professor of Physiology at Stanford University and Frank W. Weymouth, Ph.D., Associate Professor of Physiology in Stanford University. Cloth. Pp. 784. Lea & Febiger. Philadelphia, 1928.

‡Bolles Lee's Microtomist's Vade-Mecum. A Handbook of The Methods of Microscopic Anatomy. Ninth Edition Edited by J. Bronte Gatenby and E. V. Cowdry. With Nine Illustrations. Cloth. Pp. 710. P. Blakiston's Son & Company, Philadelphia, Pa., 1928.

*Lectures on Medicine and Surgery**

VOLUMES containing collections of addresses by eminent men on very practical aspects of the practice of medicine and surgery are gaining deserved recognition. As a consequence several organizations are entering this field. One of the latest is the New York Academy of Medicine. This Academy has a wealth of good material to draw upon and it has selected wisely in its contributions to its first series of lectures on medicine and surgery.

A very readable lecture on the Surgical Aspects of Medical Conditions brings to mind that another contribution of equal or even greater value would be one on the Medical Aspects of Surgical Conditions. Too often when the postoperative case goes bad the surgeon rather glibly tells the anxious relatives that the kidneys have played out or that the heart is failing when there is no clinical justification for such a diagnosis.

Out our way we speak of two types of digitalis dosage, the medical dose and the surgical dose. The surgeon too often when the pulse gets a little rapid gives a single hypodermic injection of some variant of digitalis and settles back content with the self-assurance that he has digitalized his patient. It reminds one of the man who attempts to tickle the elephant with a straw.

George M. MacKee in a lecture on The Cutaneous Manifestations of Syphilis produces one of the best series of illustrative photographs that I have had occasion to see. They remind us that the badly disfiguring and ulcerative lesions of tertiary syphilis are not yet entirely things of the past.

Dr. James Alexander Miller contributes a lecture on Climate in Tuberculosis. He discusses climatic factors such as temperature, humidity, wind, dust, storm, character of the soil, and vegetation and configuration of the country. His conclusions are worthy of repetition, that while many cases can be satisfactorily treated in or near their home environment, others actually do better in other climates and the climate for one patient is not necessarily the appropriate one for another. Many factors must be considered in the selection of the proper climate for a tuberculous individual.

Some will disagree with certain of the statements by Louis K. Neff on Useful Drugs in Clinical Practice. But this is true in nearly all such discussions for we try to generalize on our experience with individual cases, failing to realize that each is different and must be treated on its own merits.

David Riesman contributes an excellent discussion of the Treatment of Pneumonia. While we have no proved specific for pneumonia, so much has been written on fundamental principles in this disease that it would seem superfluous to reiterate them. But every consultant knows from his own experience that the average general practitioner is still treating pneumonia as it was treated thirty years ago with unjustified stimulation and improper use of digitalis. Dr. Riesman's article is deserving of a wide audience.

Clinical Case-Taking†

ONE can recognize distinct advantages in Dr. Herrmann's decision to publish this small manual as a separate and supplementary volume to his Methods in Medicine, for this is a work which the beginner in physical examination will want to have available even at the bedside and it should therefore be in a handy compact form.

Nonessentials appear to have been well eliminated and yet the details of history taking and physical examination are described well enough so that the work is quite complete for the purposes for which it is intended.

The graduate who has dropped out of the habit of painstaking examination will derive great help from its use.

---

*The New York Academy of Medicine Lectures on Medicine and Surgery. With Thirty-Nine Illustrations. Cloth. Pp. 319. Paul B. Hoeber, Inc., New York, 1928.

†Clinical Case-Taking. Supplement to Methods in Medicine. By George R. Herrmann, M.D., Ph.D. Assistant Professor of Medicine, Tulane University, New Orleans. Cloth. Pp. 90. The C. V. Mosby Company, St. Louis, Mo., 1927.

The Journal of Laboratory and Clinical Medicine

VOL. XIV ST. LOUIS, MO., FEBRUARY, 1929 NO. 5

Editor: WARREN T. VAUGHAN, M.D.
Richmond, Va.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - - - - CINCINNATI
PAUL G. WOOLLEY, M.D. - - - - - - LOS ANGELES
J. J. R. MACLEOD, M.B. - - - - ABERDEEN, SCOTLAND
W. C. MACCARTY, M.D. - - - - - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - - - - - COLORADO SPRINGS
VICTOR C. MEYERS, PH.D. - - - - - - CLEVELAND
RUSSELL L. HADEN, M.D. - - - - - ROSEDALE, KAN.
JOHN A. KOLMER, M.D. - - - - - - PHILADELPHIA
ROBERT A. KILDUFFE, M.D. - - ATLANTIC CITY, N. J.
A. PARKER HITCHENS, M.D. - - - - MANILA, P. I.
DEAN LEWIS, M.D. - - - - - - - BALTIMORE
GEORGE HERRMANN, M.D. - - - - - NEW ORLEANS
HAROLD D. CAYLOR, M.D. - - - - ROCHESTER, MINN.
T. B. MAGATH, M.D. - - - - - ROCHESTER, MINN.

Official Organ of the American Society of Clinical Pathologists and the American Association for the Study of Allergy

Contents of this Journal Copyright, 1929, by The C. V. Mosby Company—All Rights Reserved

Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIALS

A Neglected Field for Studies in Heredity

MALIGNANT neoplastic diseases are apparently becoming more significant in biology and in the practice of medicine. Many valuable observations made in the past that have advanced the knowledge of malignant diseases in man have resulted from studies of malignant tumors as they are found in animals. More extensive observations and general interest in malignant tumors as they occur in animals may further aid in the solution of some of the problems in the investigation of cancer.

Most of the data concerning spontaneous malignant neoplasms have been obtained from the smaller laboratory animals, particularly mice. Obviously domestic animals that are used for food are usually killed before they have reached the age at which neoplastic changes usually occur.

In horses and dairy cattle, however, breeding lines are known for many generations. For example, the thoroughbred line of horses was founded largely from the blood lines of three sires, Godolphin Bart, Byerly Turk and

Darley Arabian. These three horses were brought to England in 1724, 1689, and 1706, respectively. The get of this breed are almost all on record and they include thousands of individuals and a great many generations of animals. Godolphin Bart alone was the sire of more than 1200 colts. The father of the American breed of trotting horses is generally conceded to be Hambletonian X. This colt was foaled May 15, 1849, and his ancestors were known for five generations. More than sixteen generations of horses trace their lineage back to this famous sire. Intimate data regarding these animals are known, their track records, their offspring and their pedigree. The breeding lines of certain dairy cattle go back a great many generations. Milking records and breeding data are all recorded.

All of this great mass of data, which is of value to a student of genetics, is available, but an important element of economic significance and importance in human affairs is missing: that is, information regarding the kind and incidence of neoplastic diseases present in these animals. Even if new-growths are rare it would seem that data on this fact are of importance. Old pure bred cattle are usually butchered and there is no chance to follow them through the abattoir and determine the presence or absence of new-growths. Horses, however, especially if they are of racing stock, usually live to old age and die of natural causes.

Necropsy of horses of racing stock by a competent veterinarian, and the microscopic examination of any tumors found, in consideration of the great number of colts from one sire and the large mass of data constantly accumulating, might prove a fruitful source of information for the study of the influence of genetic factors in neoplastic disorders.

—H. D. C.

The Filtrable Form of the Tubercle Bacillus

EVERYONE who has searched sputum smears for hours without finding tubercle bacilli, while the same sputum readily causes tuberculosis in a guinea pig, must be anxious to believe in the possibility of the occurrence of another form of the tubercle bacillus than the common acid-fast one. The question has arisen whether this other form may be the well-known Much's granules, or whether a real "fluidum contagium vivum" exists.

Fontes[1] reported in 1910 that Berkefeld filtrates of tuberculous caseous material caused enlargement of the lymph nodes in guinea pigs. He demonstrated acid-fast organisms in these lesions. In 1922 and the following years Vaudremer[2] reported similar findings with filtrates from glycerin-free broth and potato water cultures. He believed, as Fontes did, that the tuberculous virus passes the filter in the form of Much's granules. These were formed abundantly in his cultures. When the tubercle bacillus grew under the surface in potato water it developed a nonacid-fast mycelium and numerous granules. Inoculation of the filtrate in guinea pigs caused enlargement of the lymph glands from which Vaudremer isolated tubercle bacilli of low virulence. Valtis[3] inoculated filtrates of autolysed sputum and found acid-fast organisms in the lungs but there were no swollen lymph glands. In another experiment

the filtered pus from a monkey node caused typical tuberculosis in a guinea pig. Arloing and Dufourt[4] obtained a filtrable form of low virulence from tuberculous organs which caused tuberculosis of the lymph glands, but they were unable to demonstrate the existence of a filtrable form in culture media. Nor could any of these authors confirm Vaudremer's findings of a nonacid-fast mycelium. Several other French authors obtained similar results and in this country Mellon and Jost[5] reported positive findings. On the other hand Cooper and Petroff[6] did not find any evidence of a filtrable form but observed acid-fast granules in the lymph glands of 33 per cent of their controls. Montemartini,[7] Fessler,[8] Rossetti[9] and Ruys[10] had negative results.

Fontes used diatomaceous filters (Berkefeld), but most of the other workers used porcelain filters (Chamberland L2 and L3). According to the makers[11] the size of the pores in the Berkefeld filters varies from 4 to 12 μ according to the grade, but Rosenthal[12] and Mudd[13] found that the effective diameter of the pores is much less, approximately 0.5 μ. The makers of the Chamberland filters state that the L2 filter permits small bacilli to pass and that the L3 filter retains even the diphtheria bacillus. It is evident that the mere fact that the tuberculous virus passes through these filters is not sufficient proof that we are dealing with a filtrable virus. The results would have carried more conviction if one of the denser filters like L7 had been used. The diameter of the pores is not the only factor which determines whether a given bacillus will pass through a filter. Both the filter and the bacillus carry a negative charge and only negatively charged particles will pass through a negative filter. But the p_H of the medium and the presence of polyvalent ions may change the potential of the organisms and absorption of proteins from the tuberculous exudates may change the nature of the porcelain surface. Under these circumstances it is not surprising that widely differing results were obtained by different authors, and that the experimenters who reported positive results have not been able to reproduce these results constantly. These differences may not be the result of a capricious behavior of the tubercle bacillus but may simply be the expression of our deficient knowledge of the conditions which influence filtration.

It has been suggested that the positive results can be explained by the occasional passage of an intact tubercle bacillus. This is by no means impossible. The bacterium pneumosintes of Olitsky and Gates and the Schizosaccharomyces filtrans of Lewis are frankly filter-passing. Avian diphtheria, Oroya fever, contagious pleuropneumonia of cattle and scarlet fever were at one time described as caused by filtrable viruses but are now recognized as the result of bacterial infection. The opposite has also occurred. Hog cholera was formerly supposed to be the result of infection with the hog cholera bacillus but is now considered as due to a filtrable virus.

There are, however, important reasons for not lightly dismissing the possibility of a filtrable form of the tubercle bacillus. Most of the authors who have affirmed the existence of this form report that it is usually of very low virulence and only regains its virulence after many animal passages, if at all. In experiments in which guinea pigs were infected with one single

bacillus this has not been observed. If the guinea pigs became tuberculous, the bacilli caused the usual form of disease. Vaudremer describes the symptoms caused by his filtrable virus as resembling Poncet's syndrome. Vasiliu and Iriminoiu[14] found a filter-passing form of Koch's bacillus in the glands of patients suffering from lymphogranuloma. In this relation it is interesting to remember that Falk[15] found a difference in electrophoretic potential between virulent and avirulent strains of pneumococci. A similar difference in the tubercle bacillus could influence their filtrability.

The possibility of spontaneous infection must always be considered in experiments with the tubercle bacillus. Calmette[16] states that in his experiments the acid-fast organisms were found principally in the tracheobronchial lymph nodes, but no primary effect existed at the point of inoculation. This points to the possibility of an infection by air, either by the tubercle bacillus or by an organism similar to the timothy bacillus as suggested by Cooper and Petroff.

At present it seems impossible to choose between these different possibilities and further experiments will have to decide if a filtrable form of the tubercle bacillus exists and whether Much's granules or an ultramicroscopic virus constitutes this form.

REFERENCES

1. Fontes: Memoris do Inst. Oswaldo Cruz. **2**: 1, 1910.
2. Vaudremer: Compt. rend. Soc. de biol. **89**: 80, 1923.
3. Valtis: Compt. rend. Soc. de biol. **90**: 9, 1924.
4. Arloing and Dufour: Compt. rend. Soc. de biol. **94**: 46, 1925.
5. Mellon and Jost: Proc. Soc. Exper. Biol. & Med. **24**: 743, 1927.
6. Cooper and Petroff: J. Infect. Dis. **43**: 200, 1928.
7. Montemartini: Bull. de l'Inst. sierother. Milan. **4**: 1, 1925.
8. Fessler: Centralbl. f. Bakteriol. **98**: 148, 1926.
9. Rossetti: Bull. de l'Inst. sierother. Milan. **6**: 111, 1927.
10. Ruys: Nederl. Tijdschr. v. Geneesk. **72**: 795, 1928.
11. Rivers: Filtrable Viruses, p. 86.
12. Rosenthal: Ztschr. f. Hyg. **60**: 169, 1908.
13. Mudd: J. Bacteriol. **8**: 459, 1923.
14. Vasiliu and Iriminoiu: Compt. rend. Soc. de biol. **94**: 163, 1925.
15. Falk, Gassin, and Jacobson: J. Infect. Dis. **37**: 481, 1925.
16. Calmette: L'Infection bacillaire et la Tuberculose, p. 157, 1927.

—G. B. W. (C. H. B.)

CORRESPONDENCE

To the Editor.—In an article in the November, 1928, issue of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, entitled "The Rôle of Complement in Health and Disease" by L. J. Hadjopoulos, M.D., and Reginald Burbank, M.D., from the Laboratories of the Beth Israel Hospital, New York City, the authors, after making a reference to the work of Dungern, published in 1900, make the statement in regard to complement—"Subsequent literature offers only fragmentary or indirect evidence of its rôle as a protective mechanism." Also, they make a further statement that this particular phase of the subject had been "hitherto neglected." I beg to point out that important work and observations along this line of investigation had been published by W. C. Gunn in 1914, by Hiram D. Moore, in 1919, and by Rockwood and Beeler in 1924. Moreover, the experiments, observations and conclusions of Drs. Hadjopoulos and Burbank parallel to a very considerable extent those to be found in an article entitled "Complement in Health and Disease" by the writer, published in the Canadian Medical Association Journal in April, 1926.

(Signed) FRED T. CADHAM.

Winnipeg, Canada, Dec. 11, 1928.

To the Editor.—I regret that in our article "The Rôle of Complement in Health and Disease" due credit was not given for an excellent article along similar lines by Doctor Fred T. Cadham of Manitoba, but at the time our work was first prepared for publication and read before a New York medical group, Doctor Cadham's article had not been published, and no further search of the literature was made after that time.

The work was merely presented as our research during a period of some nine years prior to 1926.

(Signed) REGINALD BURBANK.

New York City, Dec. 26, 1928.

American Society of Clinical Pathologists

Dr. F. W. Hartman, Detroit, Michigan
President

Dr. J. H. Black, Dallas, Texas
President-Elect

Dr. Charles R. Drake, Minneapolis, Minnesota
Vice-President

Dr. H. J. Corper, Denver, Colorado
Secretary-Treasurer

Dr. T. B. Magath, Rochester, Minn., *Editor*

Associate Editors

Dr. Robert A. Keilty
Washington, D. C.

Dr. Kenneth M. Lynch
Charleston, S. C.

Executive Committee:
Dr. A. H. Sanford, Rochester, Minn.
Chairman
Dr. W. W. Coulter, Houston, Texas
Dr. Herman Spitz, Nashville, Tenn.
Dr. Frederic E. Sondern, New York
Dr. Wm. G. Exton, Newark, N. J.
Dr. John A. Kolmer, Philadelphia, Pa.

Board of Censors
Dr. C. H. Manlove, Portland, Ore.
Chairman
Dr. Robert A. Kilduffe, Atlantic City
Dr. H. R. Mills, Tampa, Florida
Dr. Reuben Ottenberg, New York
Dr. Ernest Scott, Columbus, Ohio
Dr. B. W. Rhamy, Fort Wayne, Ind.

Past Presidents

Dr. Philip Hillkowitz, Denver, Colo.
1922-3
Dr. Frederic E. Sondern, New York, N. Y.
1925-6
Dr. Wm. Carpenter MacCarty, Rochester, Minn.
1923-4
Dr. Wm. G. Exton, Newark, N. J.
1926-7
Dr. John A. Kolmer, Philadelphia, Pa.
1924-5
Dr. A. H. Sanford, Rochester, Minn.
1927-8

GENERAL LETTER NO. 1

The Program Committee earnestly requests the membership of the Society to consider and plan now the papers to be presented at the coming meeting and, in order that the program may be well balanced, of general interest, and in keeping with the prestige of the American Society of Clinical Pathologists the Committee requests:

1. That all those desiring to present a paper will notify the Chairman as early as possible even if only the subject and not the title can be stated.

2. That, at least two months before the time of the meeting, five copies of the title and abstract of the paper be furnished the Chairman.

3. That, in so far as is possible, the papers represent original work of scientific or general interest.

4. That it be specifically stated whether or not lantern slides will be used.

The Committee hopes that there will be a very general response to these suggestions.

The duration of the meeting and the size of the membership make it obvious that, perhaps, not all who desire to do so will be able to present their papers. The earlier the response, however, the better will be the opportunity for a place on the program.

ROBERT A. KILDUFFE, M.D.
CHAIRMAN, PROGRAM COMM.
ATLANTIC CITY HOSPITAL
ATLANTIC CITY, N. J.

GENERAL LETTER NO. 2

Preparations are being made for a Scientific Exhibit at the Portland meeting of the American Society of Clinical Pathologists. Undoubtedly many of our members have some interesting specimens, apparatus, or technical methods that they may desire to show to the Fellows at the Convention.

We shall appreciate any cooperation on your part in making this feature of our annual meeting a source of instruction to our membership. Kindly let me know what we may expect from you in this field so that we can make the proper arrangements for its display.

E. D. Downing, M.D.
Chairman, Committee on Exhibits
Woodman Sanitarium
Woodman, Colorado.

GENERAL LETTER NO. 3

At the last meeting of the Society in Minneapolis it was unanimously agreed that there would be an annual award of a prize to be bestowed on the member of the Society who shall have made the most valuable and original contribution in the field of clinical pathology, this prize to be called "The Ward Burdick Award of the American Society of Clinical Pathologists." The nature of the award shall be decided on, and the winner selected by the Research Committee. For this year, at least, the award is to be given irrespective of the subject studied, although possibly in later years other committees may see fit to limit the field to research on certain subjects.

It is hoped that many of our members will submit their work for the consideration of the Research Committee at the same time that the papers are sent to the Program Committee so that ample time is allowed for mailing the same to the various members of the Research Committee for their perusal. No papers will be accepted from applicants later than two months before the next annual meeting in Portland. Your Committee has deemed it advisable at this time to allow any member, whether conducting a small laboratory or working in a large institution to compete for the prize, realizing as we do that the individual efforts and originality are the basis for estimation of merit.

The Committee has been considering the advisability of endeavoring to foster the study of certain conditions such as "Undulant Fever," "Agranulocytosis," "Acute Leucemia," by our members throughout the country, with the idea of suggesting to the President and the Program Committee that a half day be given over at our next meeting for papers on one of the above subjects, followed by a round-table discussion on the matter. It is suggested that by such a plan many of our members who are so situated that they cannot devote much or any time to a definite problem of research, may be able to bring in good case reports and experiences which, when collected, will be of great mutual benefit. If such plans are ultimately agreed upon it is hoped that ample photographs, slides or pathologic material will be presented for a thorough discussion of any case or cases described. Any suggestions in regard to the above method or procedure or other matter of research will be gratefully received by your Committee.

Alvin G. Foord, M.D.
Chairman of Research Committee.

THE REGISTRY OF TECHNICIANS—PROPOSED WORKING SCHEME

I. NAME

1. The Registry shall be known as The Registry of Technicians of the American Society of Clinical Pathologists, and shall be directed by a Board of Registry of six members appointed by the Society.

II. OBJECTS

1. The objects of the Registry shall be:
   a. To establish the minimum standards of educational and technical qualifications for various technical workers in the clinical, research and public health laboratories.

b. To classify them according to these standards.

c. To receive applications for registration and issue a certificate of registration to those who meet the minimum standards of requirements.

d. To register schools which offer an acceptable course of laboratory training.

e. To conduct a placement bureau for registered laboratory technicians.

f. To cultivate a high ethical standard among laboratory technicians in accordance with the code of ethics established by the American Society of Clinical Pathologists.

III. BOARD OF REGISTRY

1. The Board of Registry shall be composed of six members elected by the American Society of Clinical Pathologists, two members to be appointed by ballot to serve for three years at each annual meeting of the Society or until their successors have been elected. The first Board shall consist of six members, two of whom shall be elected for a term of one year, two for a term of two years and two for a term of three years. It shall elect its own chairman from among the holdover members and Secretary-Treasurer.

2. The Board of Registry shall be authorized to employ a director who is empowered to manage the affairs of the Board.

3. The duties of the Director shall be to administer the office of the Board by taking charge of registration of technicians, issuance of certificates, and conducting a placement bureau and such other business as may be necessary to carry out the functions of this Board. He shall be directly responsible to the Board.

IV. CLASSIFICATION OF LABORATORY TECHNICIANS

1. Technical workers in the clinical research or public health laboratories shall be classified according to their education, training and experience, as follows:

a. Medical Technologist.

b. Laboratory Technician.

2. Medical Technologist shall signify one who possesses a university degree with at least one year in basic sciences including chemistry, bacteriology, physiology and pathology with laboratory demonstration or credit equivalent to the same as determined by the Board and at least one year of practical experience in a recognized laboratory, devotes himself wholly to the technical work of a medical laboratory, and has rendered a valuable service in the field of laboratory medicine through research, teaching or other scientific endeavors. Medical Technologists shall be elected annually by the unanimous vote of the Board of Registry. A laboratory technician who possesses no college degree but who has rendered long and faithful service in a recognized clinical, research or public health laboratory in a responsible capacity, may be eligible to this designation.

3. Laboratory Technician shall signify one who is fully qualified to render general or special technical service in a clinical, research or public health laboratory under the supervision of a qualified director, and shall exhibit the following minimum preparation and qualification.

a. Graduation from an accredited high school.

b. One year of didactic work in basic sciences including chemistry, bacteriology, physiology and pathology, together with laboratory demonstration, or credit equivalent to the same as determined by the Board.

c. Six months of actual experience in a recognized clinical, research or public health laboratory.

4. Laboratory Technician or Medical Technologist who limits his work in a certain special field shall be so designated as Bacteriological Laboratory Technician, Chemical Laboratory Technician, Public Health Laboratory Technician, etc., in the case of laboratory technicians and bacteriologist, serologist, parasitologist, etc., in the case of medical technologists.

V. REGISTRATION OF TECHNICIANS: CERTIFICATES

1. Candidate shall properly fill out an application blank of the Registry and file with the Director of the Board of Registry.

2. A registration fee of three dollars shall accompany the application. This will be returned if the application is rejected.

3. Annual renewal of the certificate is required for which a fee of one dollar is charged.

4. Upon the receipt of application the Director shall conduct a preliminary investigation of each applicant and the result shall be filed with the application. Certification of applicants shall be done by the Board of Registry at the annual meeting.

5. A certificate of registration shall be issued to all applicants accepted by the Registry.

6. A certificate may be revoked at any time for cause by order of the Board. A hearing may be granted on request.

VI. EXAMINATION

1. A formal examination may be deemed necessary by the Board to determine the qualifications of an applicant in which case, written, oral and practical examinations shall be conducted at a place and by a member of this Society as arranged by the Director of the Board.

2. An additional fee of $10.00 to cover the expense shall be charged the applicant.

VII. REGISTRATION OF SCHOOLS FOR LABORATORY TECHNICIANS

1. The Board shall investigate, classify and periodically inspect through an accredited representative, the schools and laboratories which conduct a training course for laboratory technicians.

2. These schools and laboratories may register with this Board and receive an annual certificate of registration provided the course of training given meets the approval of this Board. An annual registration fee of one dollar shall accompany the application.

VIII. PLACEMENT BUREAU

1. Registered technicians and technologists may, upon proper application, be placed through this Bureau operated by the Board.

2. A fee equivalent to five per cent of the first month's salary shall be charged to the technician who obtains employment through this Bureau.

IX. CODE OF ETHICS

1. All registered technicians and technologists shall be required to strictly observe the Code of Ethics as defined by the American Society of Clinical Pathologists, namely, that they shall agree to work at all times under the supervision of a qualified physician and shall under no circumstances, on their own initiative, render written or oral diagnoses except in so far as it is self-evident in the report, or advise physicians and others in the treatment of disease, or operate a laboratory independently without the supervision of a qualified physician or clinical pathologist.

News and Notes

The Registry of Technicians of the American Society of Clinical Pathologists is now functioning quite satisfactorily considering the short period that it has been established. The aims and objects of the Registry are presented on another page.

The establishment of the Registry will prove a great boon both to technicians as well as to the hospitals and clinical pathologists. In this age of efficiency and mass production the rise and growth of this handmaiden to the physician will be of incalculable value in the saving of human lives by placing properly qualified technicians in localities where there are none at present.

Technicians are rapidly availing themselves of the opportunity to obtain certificates in the Registry, thus raising the status of their craft to an elevated and respected position.

Members will kindly report any change in address to the Secretary of the American Society of Clinical Pathologists, Metropolitan Building, Denver, Colorado.

The Service Bureau of the American Society of Clinical Pathologists has also been active in making contacts between pathologists seeking a change in location and institutions desirous of engaging their services.

The next annual meeting of the American Society of Clinical Pathologists will be held in Portland, Oregon, July 5, 6, and 8, 1929. All members are urged to make plans to attend this convention which besides promising a great intellectual feast will also enable them to visit our great far west.

The attention of the members of the American Society of Clinical Pathologists is called to the fact that at our next convention in Portland, Oregon, one-half day will be devoted to a group presentation and general discussion of the subject of "Undulant Fever." Participation in this symposium by all the members is earnestly requested by the Program Committee and Research Committee.